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SELECTION FOR SEX RATIO IN MICE AND DROSOPHILA

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INTRODUCTION

It has often been argued that sex ratio is under the control of natural selection and is thereby adjusted to an optimum value, often supposed to be equality of the sexes at the age of reproduction. (See for example Fisher, 1930, and a recent examination of the idea by Shaw and Mohler, 1953). From this idea the inference readily follows that artificial selection ought to be capable of altering the sex ratio. Experience shows that metrical characters for which natural selection appears to favor an intermediate value commonly show much genetic variability and respond readily to selection. There is no apparent reason why sex ratio should behave differently. Factual evidence about the efficacy of selection applied to sex ratio is scanty and conflicting. The work described in this paper was therefore undertaken with the object of providing a little more information on these points. The nature of the existing evidence will first be briefly indicated. (This is in no sense a complete review of the vast literature on sex ratio.)

In the first place, environmental agencies that have modified sex ratio have often been noted. Diet in mice (Bittner, quoted by Grüneberg, 1952) will serve as an example. Though such agencies probably operate for the most part through differential mortality after fertilization there is no reason why genetic variation should not become manifest through the same channels. Secondly, several genes that affect sex ratio are known in *Drosophila* species (see for example Novitski, 1947, and Gowen, 1948). If such major genes exist it is reasonable to suppose the existence also of minor genes giving rise to multifactorial genetic variability. Variability of sex ratio between different progenies, whether genetic or environmental in origin, should be revealed, if present, by appropriate statistical analysis. The evidence obtained in this way is, however, ambiguous. Howard, McLaren, Michie and Sander (in press) found significant heterogeneity be-

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tween the F_1 s of different inbred strains of mice: this variability can only have been genetic in origin. But Robertson's (1951) analysis of cattle and human families, though suggestive of the existence of some heterogeneity between families, failed to establish its significance even with very large numbers. Finally, there have been a few attempts to modify sex ratio by experimental selection, and these results—which are again conflicting—must be examined in rather more detail.

Moenkhaus (1911) claimed that his selection in *Drosophila melanogaster* had been successful, but this was before the existence of sex-linked lethals was known, and Warren (1918) pointed out that the success of the selection was probably due simply to the presence of a sex-linked lethal in one of the wild females from which the selected lines were derived. Warren (1918) claimed to have proved that sex ratio is not modifiable by selection in the absence of sex-linked lethals, since he failed to produce any change by selection in two different stocks. But Warren's selected lines were maintained by brother-sister matings and nearly always the progeny selected in each generation came from only one pair of parents. Under this mating system any genetic variation present at the beginning would be expected to be rapidly eliminated by the inbreeding, and the absence of a response to selection therefore tells us very little. The experiment by King (1918) on rats is well known. Selection in opposite directions was undoubtedly effective in producing a difference in sex ratio in the two lines. The two lines originated from a single litter of four rats, one pair for each line. The lines were maintained throughout by full-sib mating, but with retention of sublines at least up to generation 5. Selection for sex ratio began in generation 7, one line being continued from litters with an excess of males, the other from litters with an excess of females. The response was immediate and the difference between the lines increased for about five or six generations. Presumably selection operated by elimination of sublines and became ineffective when each line was reduced to a single subline. After six generations of selection the male line reached an average level of about 55 per cent of males and the female line about 44 per cent of males. The difference between the lines was about twice as great in first litters as in second litters, and the above figures refer to the average of first and second litters. It was shown by outcrosses that the female parent was chiefly responsible for the characteristic sex ratio of the line. The experiment on mice described below was planned with the hope that if King's results could be repeated it might provide an opportunity to analyze in more detail the causal mechanism of the difference in sex ratio. But this hope, as will be seen, was not realized. After the present work had started the remarkable observations of Weir (1953a) appeared. Weir produced a striking difference in sex ratio of mice by selecting not for sex ratio itself but for the pH of the blood. Two generations of selection in opposite directions produced a difference in blood-pH amounting to two thirds of the phenotypic standard deviation in the unselected population. The sex ratio at 30 days of age changed from 50 per cent ♂♂ in the unselected population to 55 per cent in

the high pH line and to 47 per cent in the low pH line. Outcross tests (Weir, 1953b) showed that, in contrast to King's results, the male parent was responsible for the characteristic sex ratio of the line.

The work now to be described consists of three selection experiments, one with mice and two with *Drosophila melanogaster*. In each case two-way selection was applied to a random bred population, families with the greatest excess of males being selected in the male strain, and of females in the female strain. (The term "strain" is used here because "line" is required later in connection with inbreeding.) In addition, the heterogeneity variance of sex ratio between families was determined. The technical details and the results of the mouse and *Drosophila* experiments will be described separately.

I. MOUSE EXPERIMENT

Selection. A random bred stock of mixed origin was used for the experiment. This stock was used also in three other experiments and much additional data on sex ratios in it was therefore available. It originated from crosses between (i) a strain derived from a cross of Goodale's and MacArthur's large strains (Falconer and King, 1953), (ii) a strain selected for high lactation (Bateman and Falconer, unpublished), and (iii) two strains carrying various color mutants, both having been previously crossed several times to the C57BL/Fa inbred strain. The stock was therefore expected to contain much genetic variability. The foundation population for the sex ratio selection (referred to as generation 0) was made up of 68 single pair matings, sib-matings being avoided. Selection in this and in succeeding generations was made in the following way.

Pregnant females were examined daily in the mornings, weekends included, and the young were sexed when found. Most of the litters were therefore less than 12 hours old when sexed. Only the living young were counted in the measurement of sex ratio. The classification of sex was checked at 12 days of age; hardly any errors in the first classification were found. Males remained with their mates all the time and females were allowed to produce a second litter if they did so in time for the second litters to be counted in the selection without too much delay. About half the females produced second litters. The second litters were discarded as soon as they were recorded and only the first litters were used in breeding. This was done in order to reduce the interval between generations. Selection was based on the simple arithmetic difference between the number of males and the number of females born (alive) to each pair of parents. This gives the best weighting according to family size (Robertson, 1951). Families were arranged in order of merit, and fifty mice of each sex were chosen from the best families in each strain. In the male strain, of course, fewer families were required to provide the fifty males than to provide the fifty females, and vice versa in the female strain. The average sex ratios in the selected families, weighted by the number of animals provided by each, are

given in table 1. The selected mice were mated in pairs, to give fifty matings in each strain. Matings of first cousins or closer were avoided.

The results are given in table 1. No difference in sex ratio between the strains selected in opposite directions appeared. When selection had been applied in this manner for two generations with no effect whatever, it was decided to continue by full-sib mating, partly in order to make the conditions more closely parallel to those of King's (1918) experiment, and partly in order to increase the additive genetic variance if any were present. Accordingly generation 3 was produced by full-sib mating among the mice selected from generation 2. The mice selected from generation 2 were derived from twenty families in each strain: therefore the fifty matings in generation 3 of each strain were grouped into twenty "lines." In making the selection among the families of generation 3 consideration was first

TABLE 1.

RESULTS OF THE MOUSE SELECTION EXPERIMENT. M AND F REFER TO THE STRAINS SELECTED FOR EXCESS OF MALES AND OF FEMALES RESPECTIVELY.

Generation	No. of families ¹		Total no. of mice ²		Total no. of males ²		Sex ratio per cent males		Standard error of sex ratio		Mean sex ratio of selected families ³	
	M	F	M	F	M	F	M	F	M	F	M	F
0	65		848		426		50.2		1.7		65.6	37.4
1	48	46	666	727	334	361	50.2	49.7	1.9	1.9	63.3	34.1
2	50	49	694	681	364	366	52.4	53.7	1.9	1.9	61.5	45.6
3	50	45	519	586	274	313	52.8	53.4	2.2	2.1	61.2	41.4
4	41	43	467	498	248	267	53.1	53.6	2.3	2.2

¹ Family = progeny of one pair of parents.

² Alive at birth.

³ Weighted according to number used as parents of next generation.

Weighted sex ratio = $\frac{\sum km}{\sum kn}$, where for each selected family k = number used as parents, m = number of males and n = number of males + females.

given to the mean sex ratio in the lines. The male and female strains were now regarded as a single population, subdivided into forty lines. From these the ten lines with the greatest excess of males were selected to start a new male strain, and the ten with the greatest excess of females to start a new female strain. Within each selected line the best families were chosen so as to give roughly equal representation of lines and to provide fifty sib-pairs in all to produce generation 4 of each strain. In the inbred generations the selection again produced no differentiation in sex ratio, and the experiment was discontinued at this stage, after four generations of selection.

This lack of response points to the conclusion that the stock used exhibited little or no additive genetic variance in sex ratio. We must now ask what is the maximum amount of genetic variance compatible with the observed result. It will be most convenient to express this as the maxi-

maximum admissible heritability under the conditions of the selection, heritability here referring to the ratio of response to selection differential when selection is between full-sib families of 14 members. The amount of selection applied is known, so we have to ask what is the maximum difference between the male and female strains that might fail to be detected on account of sampling error. For any one generation this may be taken as twice the standard error of the difference. The last generation for which the selection can be treated cumulatively is generation 3. Here the observed sex ratios differed by -0.6 percentage units (i.e. contrary to the direction of selection), and twice the standard error of this difference is 5.66 units. So the maximum admissible effect of selection is 5.06 units. The total selection differential between the strains up to generation 3 was 73.3 units; so the maximum admissible heritability is 6.9 per cent. The additional information from generations 1, 2 and 4 cannot easily be combined. These generations also failed to detect any difference, so the estimate is certainly too high, and it seems safe to set the maximum heritability compatible with the results at about 5 per cent.

Variance in sex ratio. The variance in sex ratio between families is made up of two components that can be separated by appropriate analysis. One is the error due to sampling which depends on the size of the families. The other is the heterogeneity, or real differences between families. This, if present, could be either genetic or environmental in origin. The method used to estimate the heterogeneity was the "simplified maximum likelihood" method of Robertson (1951). This method is preferable to the better known χ^2 method because it is not subject to errors from small numbers. The method consists of calculating a "score," K , and an amount of information, I , for each family. The heterogeneity variance is then estimated

by $\sigma_p^2 = \frac{\sum K}{\sum I}$, and the variance of the estimate is $\frac{1}{\sum I}$. For each family

$K = 2(d^2 - n)$, and $I = n(n-1)/2p^2q^2$, where d is the difference and n is the sum of the number of males and females in the family; p is the mean sex ratio in the population, and $q = 1 - p$. (The expression for I becomes $I = 8n(n-1)$ when the mean sex ratio is close to $\frac{1}{2}$ as it is in the present material.)

The analysis was applied to the records of four experiments made with the stock that was used in the sex ratio selection. The results are given in table 2. Altogether there are 1490 families with 18,330 individuals. Even with this very considerable mass of data the heterogeneity variance in sex ratio is less than its standard error. In this material there were several sources of environmental variation that might well be expected to contribute to the heterogeneity variance. The records extended over a period of four years giving the opportunity for seasonal effects to make themselves felt: the four experiments differed in the number of litters included in each family: and the breeding animals were selected for different characters in the four experiments (details are given at the foot of table 2).

The absence of detectable heterogeneity is therefore all the more striking and points strongly to the conclusion that there is virtually no genetic variance of sex ratio in this outbred stock.

It is of interest, for comparison with the selection experiment, to find what would be the heritability if the observed heterogeneity variance were real and all genetic in origin. The average family size in the selection experiment was 14, and the error variance was therefore $\frac{pq}{n} = 178.57 \times 10^{-4}$.

The estimate of heterogeneity variance from all the data available for this stock was 5.72×10^{-4} . If we accept the observed value as the best estimate of the heterogeneity variance and assume that it is all additively genetic, the heritability of sex-ratio under the conditions of the selection experiment would be 3.1 per cent.

TABLE 2.
ANALYSIS OF SEX RATIO IN VARIOUS MOUSE STOCKS. V IS THE ESTIMATE OF
HETEROGENEITY VARIANCE BETWEEN FAMILIES.

Stock	No. of families	No. of males	No. of ♂♂ + ♀♀	Sex ratio $= p \times 100$	ΣK	Σ	$V = \sigma_p^2 \times 10^4$	Standard error of V
Z	258	1851	3616	51.2	+ 12	435,216	0.27	15.15
EV	216	1971	3745	52.6	+ 192	540,192	3.55	13.61
J	689	2846	5380	52.9	+ 620	324,576	19.10	17.55
C	327	2998	5589	53.6	+ 364	776,304	4.69	11.35
All above	1490	9666	18,330	52.7	+1188	2,076,288	5.72	6.94
NF	211	2084	4188	49.8	- 164	963,280	-1.70	10.17
NS	191	1154	2259	51.1	+ 320	238,400	13.42	20.48
GM	391	2332	4559	51.2	+ 948	478,448	19.81	14.46

Stocks Z: Selection for sex ratio as described here: generations 0-2.

EV: No effective selection. Two litters.

J: Selection for litter size. (High, low and control combined.) One litter.

C: Selection for post-weaning growth. (High and low combined.) Two litters.

The same basic stock was used in all the above.

NF: Selection for large size.

Mostly two litters.

NS: Selection for small size.

GM: Selection for large size from cross of Goodale's \times MacArthur's strains. One litter.

The inbred generations, 3 and 4, of the selection experiment were analyzed in the same way for heterogeneity between lines, but none was found. And, finally, some other stocks were analyzed; the results, given in table 2, show again no significant heterogeneity. The absence of heterogeneity is therefore not peculiar to the stock used in the selection experiment.

To summarize—the results of the selection experiment and of the variance analyses agree in showing that there is effectively no genetic vari-

ance of sex ratio in this outbred stock. The variance analyses add the further conclusion that there is not even any detectable non-genetic variation.

II. DROSOPHILA EXPERIMENTS

Selection. The stock used is known as the Crianlarich stock and was kindly supplied by Drs. F. W. Robertson and E. C. R. Reeve. It originated from a single impregnated female caught in the wild, and had been maintained subsequently for three years by mass culture in half-pint milk bottles at constant temperature of 25°C. It was known to exhibit much genetic variability for body size (Robertson and Reeve, unpublished).

Two experiments were carried out with stock. The first was intended merely as a trial, with the object of establishing a standardized procedure. Much technical trouble from contamination by moulds was encountered and the experiment was discontinued after two generations of selection. The results of the selection, it was supposed, would be obtained again in the second experiment, which was carried out under better conditions and was almost free from this trouble. This supposition, however, proved wrong and the two experiments gave radically different results. It is therefore necessary to describe the first experiment here in spite of its imperfections. The two experiments were carried out in the same way but with one or two differences of detail. The following description refers in detail to the first experiment, points of difference in the second being mentioned at the end.

For the foundation generation (generation 0) fifty young impregnated females were taken from the culture bottle and isolated in vials, where they were allowed to lay for 24 hours. They were then transferred to fresh vials for a second laying period of 24 hours, after which they were discarded. The young flies emerging in the vials were sexed and counted daily. The products of one day's emergence—usually the second day—were set aside with the sexes separate. When emergence was practically complete the sex ratio in each family was calculated and the six families with the highest ratio were selected for generation 1 of the male strain, the six with the lowest for the female strain. Selection in the *Drosophila* experiments was based on the ratio, not on the difference. The vials were shaken over daily till no more flies emerged, when the final sex ratios were calculated. About ten flies of each sex from each selected family were then put all together in half-pint milk bottles, but with the sexes still separate, and left there to feed and mature for 2½ days. The sexes were then put together to mate and about five hours later the females were isolated in vials and the cycle repeated. Fifty females were thus set up in each generation of each strain. It was found that the flies isolated on emergence from the vials were not all virgin; it is therefore possible that some of the families were produced by full-sib matings, or, if the females mated again, that some families were of mixed male parentage.

The conduct of the second experiment differed in the following ways from the foregoing description: (i) the laying females were not transferred to second vials; (ii) the foundation generation consisted of 100 instead of 50

females, though the later generations had 50 as before; (iii) a control strain was maintained in exactly the same way, but without selection and with 20 instead of 50 females per generation.

The results of the two experiments are set out in tables 3 and 4. It will be seen that in the first experiment there was an immediate and significant differentiation between the strains selected in opposite directions, but in the second experiment no such response was obtained. The response in the first experiment seems to have been due entirely to a change in the strain selected for excess of females, the male strain having changed hardly at all. If one takes the total change in the female strain and divides it by the total selection applied, the realized heritability in the female strain comes to 41 per cent. Alternatively, one may take the divergence between male and female strains and the total selection applied to both, when the realized heritability for two-way selection comes to 24 per cent. In the second experiment the difference between the male and female strains in the last generation amounted to only 0.2 ± 1.81 percentage units, and the total selection applied to both strains was 116.20 units. The maximum admissible heritability, calculated in the manner described under the mouse experiment, was therefore 3.3 per cent. The results of the two experiments are undoubtedly discrepant; but the discussion of this discrepancy will be deferred till after the results of the variance analyses have been presented.

TABLE 3.

RESULTS OF FIRST DROSOPHILA EXPERIMENT. M AND F REFER TO THE STRAINS SELECTED FOR EXCESS OF MALES AND OF FEMALES RESPECTIVELY.

Gener- ation	No. of families		Total no. of flies		Total no. of males		Sex ratio per cent males		Standard error of sex ratio		Mean sex ratio of selected families	
	M	F	M	F	M	F	M	F	M	F	M	F
0	29		559		281		50.3		2.1		55.6	45.2
1	49	49	3121	3493	1535	1589	49.2	45.5	0.9	0.8	58.5	32.4
2	39	31	1718	937	874	402	50.9	42.9	1.2	1.7

Variance in sex ratio. In the first *Drosophila* experiment each family was reared in two vials. This gives a means of looking for environmental effects on sex ratio in the form of differences between vials within families. Unfortunately the bad culture conditions rendered all but generation 1 unsuitable for analysis. Generation 1 however was satisfactory in both male and female strains, and was subjected to a complete χ^2 analysis. The results are given in table 5. The deviation of the overall ratio from 0.5 and the heterogeneity between the two strains are highly significant. Differences between first and second vials ("vial-order"), which would reflect an effect of the age of the female parent, are negligible. Random differences between vials are also negligible, showing that environmental factors differentiating vials were absent. Differences between families, however, are highly significant, showing that in contrast with the mouse experiment there were here real differences in sex ratio between families.

TABLE 4.
RESULTS OF SECOND DROSOPHILA EXPERIMENT. M AND F REFER TO THE STRAINS SELECTED FOR EXCESS
OF MALES AND OF FEMALES RESPECTIVELY. C REFERS TO THE UNSELECTED CONTROL.

Generation	No. of families			Total no. of flies			Total no. of males			Sex ratio per cent males			Standard error of sex ratio			Mean sex ratio of selected families		
	M	F	C	M	F	C	M	F	C	M	F	C	M	F	C	M	F	C
0	45	47	96	1706	1907	2985	851	926	1457	49.9	48.6	48.8	1.2	1.1	0.9	66.1	33.4	
1	49	46	20	2898	1938	809	1494	969	359	51.5	50.0	44.4	0.9	1.1	1.7	59.8	37.0	
2	48	50	16	2112	2870	1019	1006	1445	491	47.6	50.7	49.9	1.1	0.9	1.6	62.9	37.7	
3	46	48	19	2365	2363	940	1166	1188	452	49.3	50.3	48.1	1.0	1.0	1.6	59.3	41.0	
4	43	49	17	1196	2105	424	595	1043	225	49.7	49.5	53.1	1.4	1.1	2.4	59.7	42.7	
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TABLE 5.

 χ^2 ANALYSIS OF GENERATION 1 OF FIRST DROSOPHILA EXPERIMENT.

Source of variation	d.f.	χ^2
Deviation from $p = 0.5$	1	21.4*
Between strains	1	11.6*
Between vial-orders	1	0.1
Strain \times vial-order interaction	1	0.5
Between families within strains	83	148.8*
Between vials within families	83	89.6

* $P < 0.001$.

The heterogeneity variance in sex ratio between families in all generations of both *Drosophila* experiments was estimated by the method applied to the mouse experiment. The male and female strains of the first experiment are treated separately on account of the significant difference of mean. Generation 0 of the first experiment is omitted because the numbers were small and it does not properly belong with either selected strain. The results are given in table 6. The estimates of heterogeneity variance accord well with the results of selection. The male strain of the first experiment and both strains of the second show small and insignificant amounts of heterogeneity. But the female strain of the first experiment, which alone responded to selection, shows a much greater amount of heterogeneity amounting to 7.5 times its standard error and therefore clearly significant. If the observed values are taken to be the best estimates of the heterogeneity variance, and this variance is assumed to be all additively genetic, we get, as explained under the mouse experiment, estimates of the maximum admissible heritability. They are 50 per cent in the female strain of the first experiment and 5.5 per cent in both strains together of the second, the average family sizes being 57 and 43 respectively. It will be remembered that the female strain of the first experiment showed a realized heritability of 41 per cent. It must be concluded that in the second *Drosophila* experiment, as in the mouse experiment, there was virtually no variance in sex ratio either genetic or non-genetic; but in the female strain of the first *Drosophila* experiment there was a considerable amount of variance most of which was genetic.

TABLE 6.

HETEROGENEITY VARIANCE BETWEEN FAMILIES IN THE TWO DROSOPHILA EXPERIMENTS.

Expt.	Strain	Total no. of flies	Total no. of males	Sex ratio $= p \times 100$	ΣK	ΣI	$V =$ $\sigma_p^2 \times 10^4$	s. e. of V
First	Male	4839	2409	49.78	+ 236	2,725,840	0.9	6.06
First	Female	4430	1991	44.94	+12,804	2,927,464	43.7	5.84
Second	Both	28,557	14,135	49.50	+ 4,008	11,814,432	3.4	2.91

The different results obtained in the two experiments can only have been due to the presence in the first of one or a few genes that were not present in the second. If the variation in sex ratio found in the first experiment were due to many genes it would hardly be possible to draw a second large sample from the same population and find no genetic variation in it. The presence of a sex-linked lethal in the first experiment seems to be a satisfactory hypothesis to account for the difference: it is consistent with the facts and is not unduly improbable. Thus, only the strain selected for excess of females changed in sex ratio, and no individual family showed a ratio inconsistent with either a 1:1 or a 1:2 ratio. The expected frequency of lethal-bearing females in an equilibrium population is about 0.8 per cent, taking the total spontaneous mutation rate of sex-linked recessive lethals to be 0.2 per cent. So it is not unreasonable to suppose that one of the fifty foundation females was heterozygous for a sex-linked lethal.

DISCUSSION

The conclusions that emerge from this and previous studies of variability of sex ratio are: sex ratio can be modified by environmental agencies, and genes that modify sex ratio exist; but no multifactorial genetic variability is detectable either by selection or by statistical analysis. In consequence selection for sex ratio may sometimes be effective, when the initial population happens to contain a major gene that affects sex ratio. The success of King's (1918) selection in rats must be ascribed to the presence of such a gene, as has already been suggested by Lush (1945). The same applies to Weir's (1953a) selection for blood-pH, where according to Weir and Clark (in press) only a few genes were responsible for the variation in blood-pH and so presumably also in sex ratio. But genes with detectable effects on sex ratio are not frequent, and in their absence selection for sex ratio is ineffective.

Thus sex ratio appears to differ from the better known "quantitative characters" such as body size or bristle number, which show much additive genetic variance in random bred populations and respond readily to selection. On the practical level, or in terms of utilizable genetic variation, this difference is real enough. But sex ratio differs in another respect from the characters commonly studied in quantitative genetics, and that is in the sampling variance. The variation we are interested in is that of the several mechanisms by which we suppose sex ratio might be modified—differences in the production, viability or motility of the sperm, and differences in the viability of the zygotes. Superimposed on whatever variation in these mechanisms that may exist is variation due to sampling, which arises from the chromosome mechanism of sex determination. This is extrinsic variation, akin to the errors of measurement in metrical characters, and it does not properly belong to the mechanisms in which we are interested. With most metrical characters commonly studied the errors of measurement are very small in relation to the real variation, and can safely be neglected. But with sex ratio the error of measurement almost completely

obscures the real variation. There may be quite a large amount of real variation, though we cannot prove it significant. Thus, the estimates of heterogeneity variance from the mouse stock and the second *Drosophila* experiment correspond to standard deviations of 0.024 and 0.018 respectively; or, in percentage units by which sex ratios have been expressed throughout this paper, 2.4 and 1.8. This seems quite a large amount. If a fair proportion of it were additively genetic and it were not obscured by the sampling variance selection would produce quite a rapid response. But when the sampling variance is present we can neither detect a response to selection nor prove that any real variation exists. If the difficulty of measurement could be overcome and we could analyze the real variation we might find the genetic situation governing modifications of sex ratio no different from that of other quantitative characters. As things are we still fail to make contact with the phenomena we seek to study. Only on the practical level is the situation now clear; the amount of multifactorial genetic variation in sex ratio is not enough to make artificial selection effective in organisms such as mice or *Drosophila*.

SUMMARY

1. Selection for sex ratio in mice was ineffective. The heritability of sex ratio under the conditions of the experiment was not above about 5 per cent.

2. Statistical analysis of sex ratio in large numbers of mice of several stocks revealed no significant heterogeneity between families within stocks.

3. Selection for sex ratio in *Drosophila melanogaster* was effective in one strain selected for excess of females. In a second experiment selection was not effective in either direction. The presence of a sex-linked lethal in the first experiment is postulated. The heritability in the second experiment was not above 3 per cent.

4. There was significant heterogeneity of sex ratio between families in the first *Drosophila* experiment but not in the second.

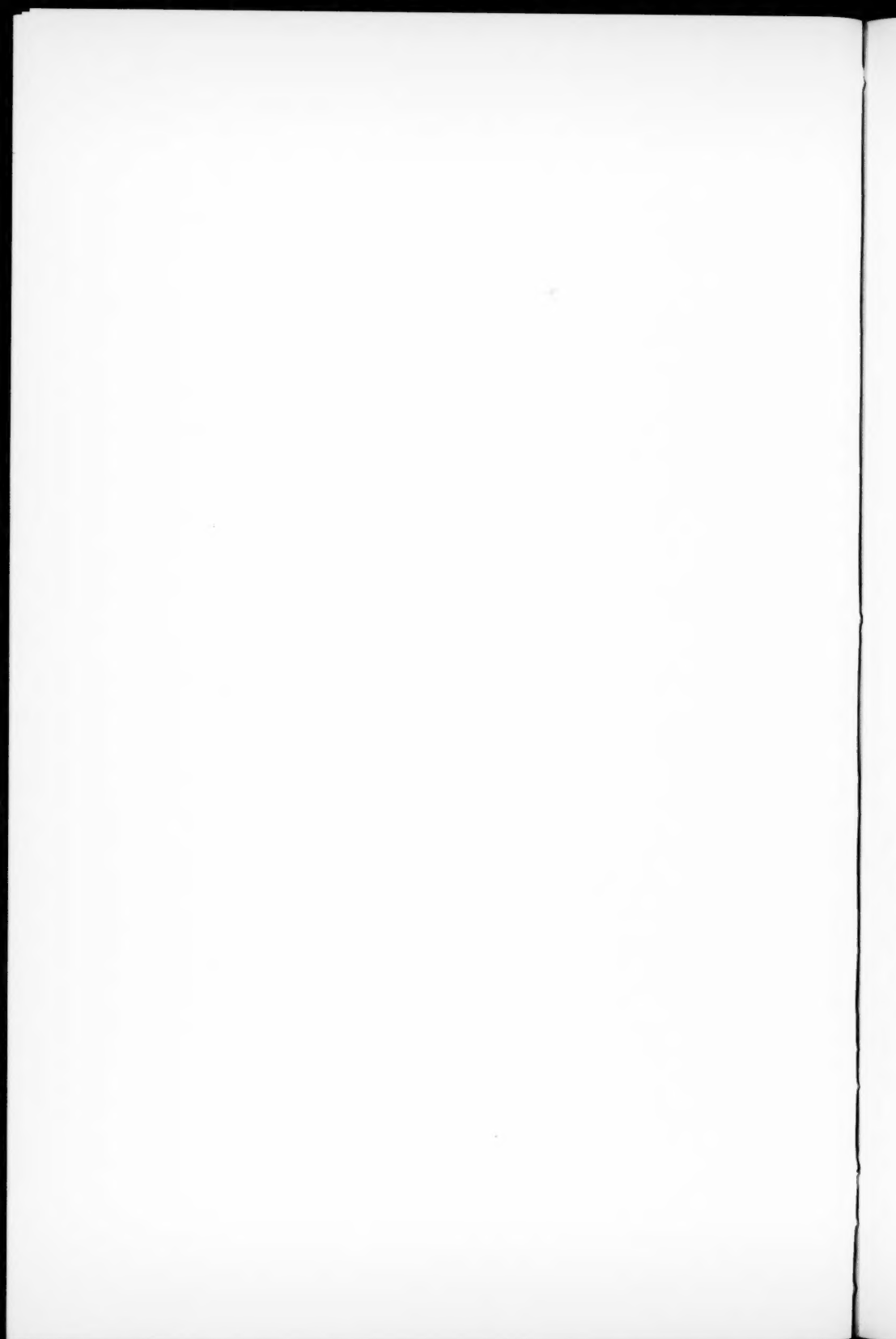
5. The absence of detectable multifactorial genetic variance affecting sex ratio suggests that sex ratio may differ from better known metrical characters, such as body-size and bristle number, in the underlying genetic control. But this difference, it is suggested, need not be real. The large amount of error variance inherent in the measurement of sex ratio misleads one into expecting a commensurate amount of real variation, and we have no logical criterion for deciding how much real variation to expect.

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CHROMOSOMAL RELATIONS IN TWO SPECIES OF *DROSOPHILA*

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The *guarani* group of *Drosophila* is composed of six closely related species, that can be separated, according to King (1947b), in two subgroups: the *guarani* subgroup, which contains *D. guarani* Dobzhansky and Pavan, 1943a, *D. guaru* Dobzhansky and Pavan, 1943a, and *D. subbadia* Patterson and Mainland, 1943; and the *guaramunu* subgroup which contains *D. guaramunu* Dobzhansky and Pavan, 1943a, *D. griseolineata* Duda, 1925b and *D. guaraja* King, 1947a. It is possible that *D. ornatifrons* Duda, 1925b, also may be a member of this group (Patterson and Stone, 1952), but this is not certain.

In recent years the six species known to belong to the *guarani* group have been subject to considerable study, mainly with respect to cytology and sexual isolation. King (1947a) made a comparative analysis of the mitotic and salivary chromosomes of these species and of interspecific hybrids from the crosses *D. guaru* \times *D. subbadia* and (*D. guaru* \times *D. subbadia*) \times *D. guarani*. His studies (King, 1947b) of morphology, sexual isolation and hybridization between the different species of the group led him to establish the two subgroups and to conclude that species of the *guarani* subgroup are much less differentiated from each other than are the species of the *guaramunu* subgroup. While he was able to obtain interspecific insemination in all types of crosses between species of the *guarani* subgroup—except between *guaru* females and *guarani* males—King was unable to obtain any interspecific insemination between species of the *guaramunu* subgroup.

Brncic (1953) published a map of the salivary chromosomes of *D. guaramunu*, and described 19 inversions in the species. In their work on the frequency of inversions in natural populations of certain South American species of *Drosophila*, Da Cunha, Brncic and Salzano (1953) found five different inversions in *D. griseolineata* and the 19 inversions previously described by Brncic in *D. guaramunu*. These populations were mostly from the state of São Paulo, with some samples from the state of Rio Grande do Sul. In a continuation of these studies, using only material from Rio Grande do Sul, twelve new inversions have been discovered in *D. guaramunu*, bring the total for this species to 31 known inversions. These twelve newly found inversions, as well as the cytology of larval *guaramunu-griseolineata* hybrids obtained from a single *D. guaramunu* female inseminated in the wild, are described in the present paper.

The quantitative data on the frequencies of inversions, jointly with ecological data will be given in another publication.

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MATERIALS AND METHODS

The salivary chromosomes of the F1 larvae from females collected in the localities of Emboaba, Bexiga, Feliz, Ponta Grossa and Muitos Capões in the state of Rio Grande do Sul were examined in order to detect any inversions present. All slide preparations were made using the regular aceto-orcein smear technique. The identification of the chromosomes of *D. guaramunu* was made with the aid of Brncic's map, which was also used in the localization of the newly discovered inversions.

THE CHROMOSOMAL COMPLEMENT OF *D. GUARAMUNU*

The metaphase plate of the mitotic chromosomes of *D. guaramunu*, as shown by Dobzhansky and Pavan (1943a) and King (1947a), is composed of five pairs of rods and one pair of dots. In the cells of the larval salivary glands, one small and five long chromosomal strands radiate from the heterochromatic chromocenter.

As pointed out by Brncic (1953) the X chromosome of this species possesses large quantities of interstitial heterochromatin that interfere with normal pairing. For this reason the chromosome usually is unspread and greatly entangled in most preparations; therefore, it has not been possible to detect inversions in it. The other chromosomes usually present no such difficulties.

All of the twelve newly discovered inversions are in chromosome IV.

DESCRIPTION OF THE NEW INVERSIONS

Nn: Sub-terminal inversion. Includes part of section 60, sections 61, 62 and almost all section 63. Found in the localities of Emboaba, Bexiga and Feliz. Very similar to inversion Aa; distinguishable because it includes section 63 (fig. 1, no. 1).

Oo: A long inversion, that includes part of section 60, the sections from 61 to 68 and part of section 69. Found only once, in the locality of Feliz, associated with inversion Ee; these two inversions are overlapping (fig. 1, no. 2).

Pp: A short inversion. Includes sections 61 and 62. Very similar to inversion Bb; distinguishable because it does not include section 63. Found only once, in Bexiga (fig. 1, no. 3).

Qq: Includes part of section 61, sections 62 and 63 and part of section 64. Found in the localities of Bexiga and Feliz (fig. 1, no. 3).

Rr: A long inversion, that includes part of section 61, the sections from 62 to 66 and part of section 67. Found only once, in the locality of Bexiga, in overlapping with inversion Ee (fig. 1, no. 5).

Ss: A short inversion. Includes sections 62 and 63. Very similar to inversion Bb; distinguishable because it does not include section 61. Found in the localities of Bexiga and Feliz (fig. 1, no. 6).

Tt: Includes sections 62, 63 and 64. Found in the localities of Emboaba, Bexiga and Feliz (fig. 1, no. 6).

Uu: A relatively long inversion, that includes part of section 62, the sections from 63 to 67, and part of section 68. Found singly in Feliz and in overlapping with inversion *Ee* in Ponta Grossa (fig. 1, no. 8).

Vv: Includes the sections from 63 to 66 and part of section 67. Found only once, in the locality of Emboaba (fig. 1, no. 9).

Ww: Includes sections 63, 64 and 65. Very similar to inversion *Tt*; easily distinguishable by more distal position of *Ww*. Found in Emboaba and Feliz (fig. 1, no. 10).

Xx: Includes sections 64, 65 and 66. Found in Emboaba, Bexiga and Feliz. Very similar to *Dd*; distinguishable because it does not include sections 67 and 68 (fig. 1, no. 11).

Yy: Includes sections 70, 71, 72 and 73. A relatively common inversion found in Emboaba, Ponta Grossa, Bexiga and Feliz, always associated with inversions *Gg* and *Hh*, forming a complex of three overlapping inversions.



FIGURE 1. Location of newly discovered inversions in chromosome IV of *Drosophila guaramunu* (Base map redrawn from Brncic, 1953).

THE SALIVARY CHROMOSOMES OF THE HYBRID BETWEEN *D. GUARAMUNU* AND *D. GRISEOLINEATA*

When the salivary glands of the larval offspring of a *D. guaramunu* female from Emboaba were examined, it was discovered that these larvae were *guaramunu-griseolineata* hybrids, the *D. guaramunu* mother having been inseminated in nature by a *D. griseolineata* male. Because of certain morphological similarities it had been previously concluded in the intraspecific studies that the *D. griseolineata* chromosomes designated II and III by Da Cunha, Brncic and Salzano (1953) are homologous with *D. guaramunu* chromosomes designated respectively as IV and V in Brncic's map. These homologies were confirmed by the hybrid pairing relationships, synapsis being between the chromosomes herein reported as being homologous. Due to these relationships, the designations of the *D. griseolineata* chromosomes is now changed to correspond with those of their *D. guaramunu* homologues.

In the hybrids the salivary chromosomes generally appeared as follows:

II Chromosome: Unpaired from middle distally to end. In one slide it was possible to distinguish inversion *Aa*, that occurs as an intraspecific arrangement in *D. guaramunu*. In a general way, pairing was loose in all regions of the chromosome.

III Chromosome: Inversions *Aa* and *Bb* were identified; they occur also as intraspecific arrangements in *D. guaramunu*. Pairing loose in all regions of the chromosome.

IV Chromosome: Only three regions paired. Unpaired regions morphologically quite dissimilar (fig. 2).

V Chromosome: In the basal region there was an inversion complex including three or more inversions. Pairing loose.

X Chromosome: Too high heterochromatic and tangled to permit analysis.

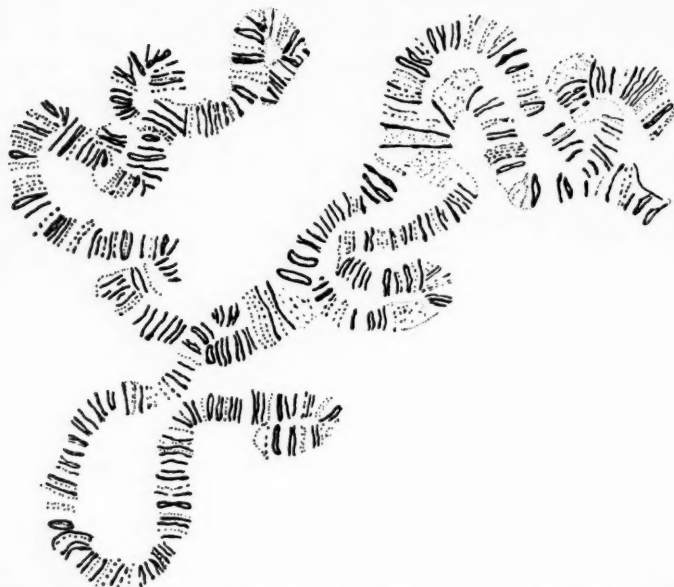


FIGURE 2. Chromosome pairing in the fourth chromosome of salivary gland cells from a hybrid between *D. guaranunum* and *D. griseolineata*.

Thus, in addition to considerable interspecific divergence in the structure of the fourth chromosome and more or less extensive lack of pairing in all chromosomes, the species hybrids show at least six inversions, three of which are known as intraspecific arrangements in *D. guaranunum*, the other three probably being entirely due to interspecific differences in the gene orders.

DISCUSSION

The analysis of 1,341 individuals of *D. guaranunum* from several natural populations in the state of Rio Grande do Sul brings to 31 the known number of different inversions possessed by this species. Thus, with respect to gene arrangements, it is one of the most highly polymorphic of *Drosophila* species. In fact, it is exceeded only by *D. willistoni*, with 44 inversions (Da Cunha, Burla and Dobzhansky, 1950; Da Cunha and Dobzhansky, unpublished; Townsend, 1952 and unpublished) and *D. paulistorum* with 34 inversions (Dobzhansky, Burla and Da Cunha, 1950). Furthermore, all 31 of the different inversions of *guaranunum* are present in natural populations of a

relatively restricted geographic region, the state of Rio Grande do Sul. The only other such high concentration of different genic arrangements has been found by Da Cunha et al. (1950) in *willistoni* in an area of comparable extent in the state of São Paulo. There the *willistoni* populations possessed 32 different inversions, or only one more than the *guaramunu* of Rio Grande do Sul. Among the different species of *Drosophila* that may show chromosomal polymorphism, some, such as *D. willistoni* and *D. paulistorum* (Dobzhansky et al., 1950) have inversions about equally distributed among their different chromosomes, while others, such as *D. pseudoobscura* (Dobzhansky and Epling, 1944), *D. athabasca* (Novitski, 1946), and *D. melanica* (Ward, 1952) have a disproportionately large number of inversions localized in one or more chromosomes. *D. guaramunu* belongs to the second class, since the number of inversions in each of its chromosomes is: II-1; III-2; IV-25; V-3.

The location of 25 inversions in a single chromosome is very remarkable. Indeed, the species that are closer to *D. guaramunu* in this particular are *D. pseudoobscura*, *D. athabasca* and *D. melanica*, each with 16 inversions in their most variable chromosome, a number very considerably less than that found in *guaramunu*. Notice must also be taken of the fact that the 25 inversions described in chromosome IV of *guaramunu* are not distributed at random along the chromosome. There is a concentration of these inversions in its basal half (from section 60 to section 69), where 17 of the 25 inversions are located. Since studies have shown that chromosomal breaks obtained by radiation or chemical treatment are distributed at random, an extreme localization such as that presented by *D. guaramunu* must be due to genic, rather than mechanical factors.

In contrast to *D. guaramunu*, *D. griseolineata* has relatively few different gene arrangements. Although 356 individuals from several natural populations of Rio Grande do Sul were examined not a single new inversion was found. The number of inversions in this species accordingly remains at five.

It is notable that *D. guaramunu* is a relatively common species at all collecting sites in Rio Grande do Sul. On the other hand, *D. griseolineata* is usually present in low frequencies, although sometimes it is rather frequent in Emboaba and Ponta Grossa.

Several workers have studied the salivary glands chromosomes of interspecific hybrids in *Drosophila* (reviews in Dobzhansky, 1951, and Patterson and Stone, 1952). Thus, Pattau, Kerkis and Horton studied the chromosomes of the hybrids between *D. melanogaster* and *D. simulans*; Tan and Dobzhansky the hybrids between *D. pseudoobscura* and *D. persimilis*; King those of the hybrids from the crosses *D. guaru* \times *D. subbadia* and (*D. guaru* \times *D. subbadia*) \times *D. guarani*; and Patterson and coworkers the hybrids between *D. virilis*, *D. americana*, *D. americana texana* and *D. novamexicana*. The study of the salivary glands chromosomes of *guaramunu-griseolineata* hybrid larvae confirms in most respects the data obtained through the intraspecific analysis; thus, the later studies show that chromosomes IV and V are most

variable with respect to the order of their genes, i. e., nearly all known inversions in *guaramunu* and all in *griseolineata* are confined to these chromosomes; while similarly the salivary chromosomes of their interspecific hybrids show that the greatest amount of interspecific structural change has occurred in the same two chromosomes. On the other hand, chromosomes II and III appear to have differentiated primarily by non-structural changes in the species. This is in spite of the fact that some inversions—all known as intraspecific gene arrangements in *guaramunu*—were found in these two chromosomes in the hybrids. While lack of pairing existed to a greater or lesser extent in all the other regions of these two chromosomes, there were no visible differences between the bands and interbands of the homologous strands. No small undefined differences, like those in the hybrids between *D. melanogaster* and *D. simulans* are present.

King (1947b) in his studies on sexual isolation was unable to obtain any interspecific insemination in the *guaramunu* subgroup. But the insemination of a *guaramunu* female by a *griseolineata* male in nature, at Emboaba, shows that sexual isolation between these species is by no means complete. In this respect the Emboaba strains apparently differ from the state of São Paulo and the Federal District strains tested by King. While the difference is likely to be intrinsic, it is not possible at present to rule out secular changes or differences in the environment as causes leading to interspecific mating. Perhaps it should be noted that *griseolineata* outnumbered *guaramunu* more than five to one in the collection in which the mother of the hybrids was taken.

Although the *guaramunu-griseolineata* hybrid larvae pupated, no imagos emerged. Thus these species are reproductively isolated from each other by at least two mechanisms, sexual isolation and hybrid inviability. When the first of these fails, the second effectively prevents gene exchange between the species and thereby maintains the integrity of each.

ACKNOWLEDGEMENTS

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SUMMARY

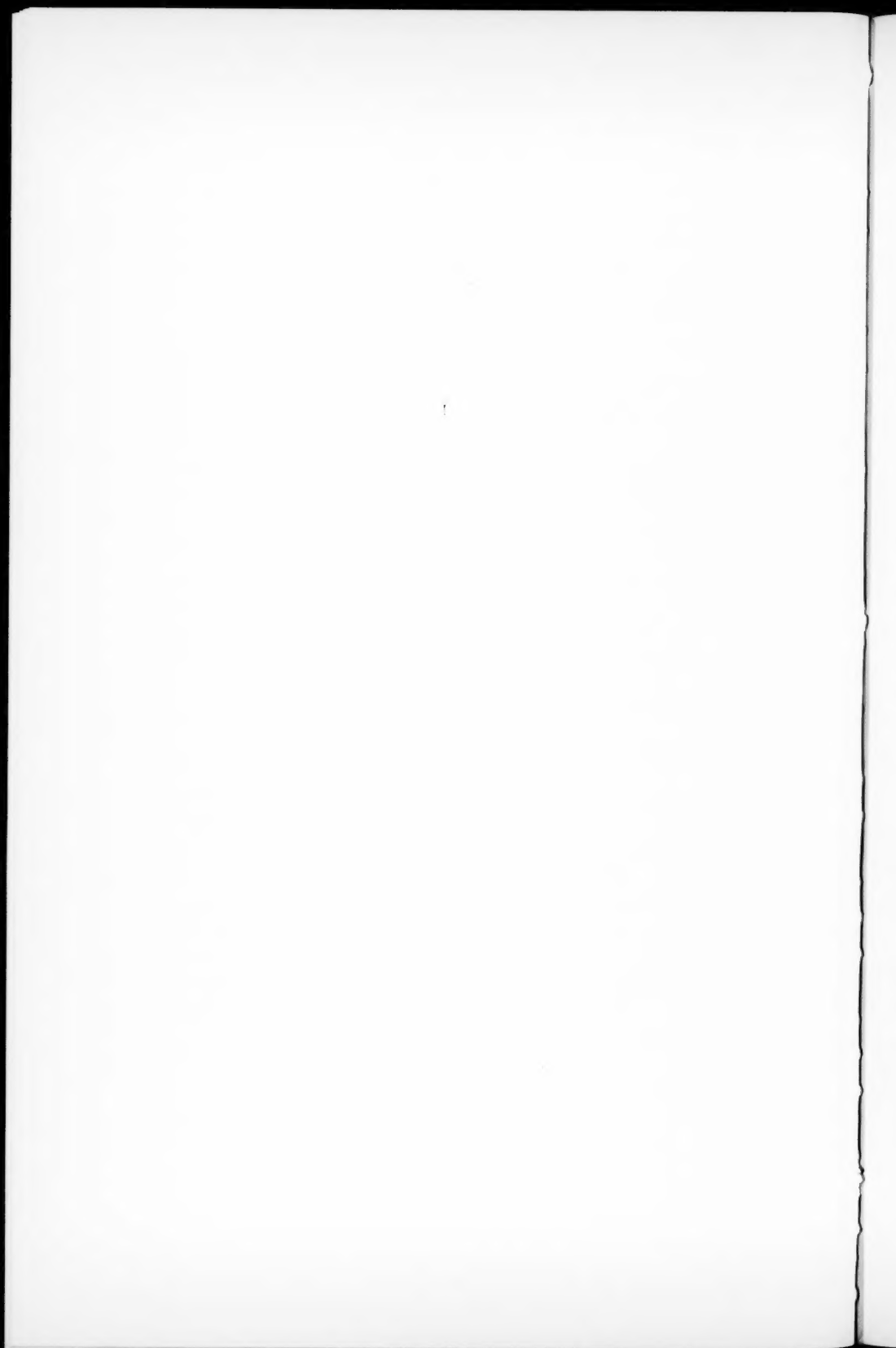
The analysis of 1,341 individuals of *D. guaramunu* and 356 of *D. griseolineata*, all from natural populations of the state of Rio Grande do Sul, Brazil, shows that the former species is highly polymorphic with respect to gene arrangements in its chromosomes, while the latter species is not. Thus the 12 new inversions herein described in *D. guaramunu* brings to 31 the

total number of inversions known in this species; yet the total for *D. griseolineata* remains at five, no new ones having been found. It is notable that 25 of the inversions of *D. guaranunu* occur in chromosome IV and 17 of these are localized in its basal half.

Hybrid larvae, but no imagos, were obtained from a *D. guaranunu* female inseminated in nature by a *D. griseolineata* male. The salivary glands chromosomes of the hybrids confirmed that the greatest divergence by structural modification has occurred between chromosomes IV and V.

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THE TOLERANCE OF *GOSSYPIUM HIRSUTUM* FOR DEFICIENCIES AND DUPLICATIONS¹

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In three earlier papers (Brown, 1949; Menzel and Brown, 1952; Menzel, in press) the unusual behavior of a reciprocal chromosome translocation (designated 2B-1) in *Gossypium hirsutum* has been described. In this line, a short distal segment of a chromosome in the D_h subgenome was interchanged with most of a long arm of a chromosome in the A_h subgenome. At metaphase I in the translocation heterozygote, an interstitial chiasma is formed 95-98 per cent of the time proximal to the point of attachment of the long translocated arm, while the short translocated arm is never paired. As a result, a ring of four is never formed, and the characteristic configuration is an association of four chromosomes composed of a ring of two having a long single side arm paired with a very unequal chain of two. The fact that each of the four chromosomes could be recognized made it possible to establish that a wide array of viable and fertile deficiency-duplication genotypes is recovered from the heterozygous translocation, each of which is cytologically identifiable.

The cytological recognition of deficiency-duplications, and the high frequency with which they were recovered, should greatly facilitate the assignment of marker genes to their respective chromosomes. Tests so far, however, with ten marker genes (R_1 , R_2 , L^0 , K , N , frego bract, cup leaf, virescent yellow, crinkled dwarf, and round leaf) have shown only that none of them is on either of the translocated chromosomes of 2B-1. An earlier suggestion (Menzel and Brown, 1952) that R_1 was located on the D_h chromosome proximal to the point of breakage was not confirmed by subsequent tests. The cytological features of the 2B-1 translocation have provided a method of attack on a number of cytogenetic problems, including analysis of the behavior and differentiation of corresponding chromosomes from related species when introduced into *G. hirsutum* (Menzel, in press).

The possibility of extending the methods of analysis used in the 2B-1 studies to other chromosomes of the *hirsutum* genome depends upon whether other translocation lines having some or all of the same features can be induced. The present paper reports the results of a preliminary experiment which had the following objectives: (1) To find an effective X-ray treatment for producing translocations and other chromosomal aberrations in cotton. (2) To locate new lines in which particular chromosomes could be identified cytologically at metaphase I by means of structural changes. (3) To recover aberrations for the chromosomes carrying the five marker genes present in

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the parent stock. (4) To estimate what proportion, if any, of the translocations induced would give rise to viable, fertile deficiency-duplications.

Before describing the experimental results, it is necessary to consider the criteria which were used for interpreting the data from observations of metaphase I in the irradiated material.

THEORETICAL CONSIDERATIONS

Configurations formed by translocation heterozygotes at metaphase I. The types of configurations formed at metaphase I in a plant heterozygous for a reciprocal interchange between two chromosomes depend primarily upon three factors: (1) the chiasma frequency characteristic of the line; (2) the relative lengths of the arms in the normal chromosomes, *i.e.*, whether the centromeres are median or subterminal; and (3) the positions of the two points of breakage and reunion, *i.e.*, their distance from the centromere and the relative lengths of the two interchanged arms.

The chiasma frequency at metaphase I is low in *G. hirsutum*, the typical bivalent having one chiasma in each arm. The single chiasma is usually terminal or nearly so. Because of the small size and compactness of the bivalents, it is often difficult to determine, when a chiasma is not clearly terminal, whether or not a second chiasma is present in the arm. However, the low frequency of arms with more than one chiasma is partially compensated by the not infrequent occurrence of bivalents with a chiasma in only one arm. The average chiasma frequency at metaphase I cannot be much above 1.0 per bivalent arm, and is probably slightly lower. While the possibility cannot be excluded that some arms characteristically have a lower or higher frequency, it can be assumed without introducing a grave source of error into the present discussion that each of the arms has a chiasma frequency of about 0.95 to 1.0 at metaphase I. It is further assumed that the chiasmata are formed at random along the whole length of the arms, except in the extreme distal and proximal segments.

According to the idiogram of the mitotic chromosomes given by Wouters (1948), which is in substantial agreement with unpublished data of Menzel and M. Ali, centromeres are mostly median or submedian in *G. hirsutum*. Only three of the 26 chromosomes in the haploid set have long arms between two and three times as long as the short arms. In these three, the short arms are as long as some of the arms of the median chromosomes. (The configurations at metaphase I in heterozygous 2B-1 suggest that one of these three chromosomes is the A-genome chromosome involved in that translocation.)

In general then, the types and frequencies of metaphase I configurations in translocation heterozygotes in *G. hirsutum* will depend mainly upon what proportions of the respective chromosome arms have been interchanged. Thus, if three-fourths of a chromosome arm has been translocated, we may expect a chiasma to be present in it in roughly 75 per cent of the pollen mother cells. Moreover, the frequency of chiasmata in the translocated segment should be inversely related to the frequency of interstitial

chiasmata between the point of breakage and the centromere of its parent chromosome. The combined frequency should equal approximately .95 to 1.0. It will be lower if the presence of translocations interferes with chiasma formation. Also, the complete failure of chiasmata to form at two positions in the 2B-1 translocation (in over 1500 cells examined so far) suggests either that the extreme proximal and distal segments do not normally form chiasmata, or that when the length of these segments falls below a certain minimum in rearrangements, chiasmata are prevented from forming in them.

G. hirsutum is an amphidiploid species with the genome formula $(AD)_4$ (Beasley, 1940). The subgenomes are referred to separately as D_h and A_h . The D_h chromosomes are on the whole somewhat smaller than the A_h chromosomes at metaphase. The configurations in the heterozygote will thus be somewhat altered by the size of the chromosomes participating in a translocation, which may either enhance or obscure differences in the proportional length of interchanged arms. Since there is some overlapping in chromosome size between the two genomes, it is not always possible to assign the participating chromosomes to either the large or the small class, and even when this can be done, the size differences serve only as an indication, not as final proof, of the genome affinities of the chromosomes involved.

Figure 1 shows diagrammatically the characteristic configurations expected in the heterozygote if (a) both breaks occurred near the centromere, (b) one break occurred near the centromere and one near the end of an arm, and (c) both breaks occurred near the end, assuming that the participating chromosomes had median or submedian centromeres. In figure 1, columns I, II, and III, is shown the effect upon the configurations if (I) both chromo-



FIGURE 1. Diagram showing the configurations expected at metaphase I in various types of translocation heterozygotes. a: both breaks near the centromere. b: one break near the centromere and one break near the end. c: both breaks near the end. I: both chromosomes large. II: both chromosomes small. III: one chromosome large and one small. In bI, bII, and bIII, the black configurations indicate the expected modal configuration, and the white configurations the (rare) maximum chiasma formation expected.

somes were large, (II) both chromosomes were small, (III) one were large and one were small.

If both breaks were either very near the end or very near the centromere, the heterozygote would be expected to show a high preponderance of cells with the same type of configurations, which would correspond to one of the types shown in figure 1. As the position of the breaks approaches the middle of the chromosome arms, the configurations will become more variable, and more different types will be encountered in the same plant. The variability of configurations in the heterozygote may thus serve as a further criterion for estimating the position of the breaks.

Identifying deficiency-duplications recovered from the heterozygote. When a line heterozygous for a reciprocal translocation (the X_1 parent in this instance) is outcrossed to a normal line, six types of numerically balanced zygotes are possible: normal, heterozygous, and four deficiency-duplications, of which two are deficient-duplicate for the centromere regions and unbroken arms, and two for the translocated ends. The feasibility of distinguishing these six types from each other at metaphase I depends upon the relative lengths of the interchanged arms and the relative sizes of the chromosomes participating in the heterozygous configuration.

While not all heterozygotes form rings, only heterozygotes can do so. Hence in any line in which some plants with associations of IV form rings and others do not, the former can be considered to be heterozygotes, and the latter, deficiency-duplications. The various deficiency-duplications cannot be distinguished unless there is a size difference as in figure 1, aIII. In many instances, the deficiency-duplications form IV's less frequently than the heterozygote, and when the IV is formed, it may show a higher frequency of interstitial chiasmata, due to the formation of two chiasmata in the duplicated arms.

In translocations between unequal arms (figure 1b), in which the heterozygotes seldom or never form rings, but do form interstitial chiasmata, it is often possible to distinguish the six genotypes visually, though with greatest ease when the inequality is augmented by a size difference between the two parental chromosomes, as in figure 1, bIII-left. (The 2B-1 translocation was of this type.) The problem is somewhat simplified, however, by the fact that if the heterozygote shows the orientation of figure 1b in a high proportion of cells, disjunction will be such that only the two deficiency-duplications for the interchanged ends will be recovered frequently. These two are rather easily identified, one by the presence in most cells of 26 II, of which one is open and more or less unequal, and the other by a IV which resembles the heterozygote (though it may be formed less frequently) except that the free end is larger.

When both segments are very short, it may be difficult or impossible to distinguish the heterozygote from deficiency-duplication types, or even from normal plants at metaphase I. If both segments behave like the shorter segment of 2B-1, the reciprocal translocation would give only bivalents in the heterozygote, and would be undetectable cytologically. It is likely that

some of the deficiency-duplications for such short interchanged arms would be functional, in which case they could be detected only by genetic evidence.

In addition, deficiency-duplications resulting from 3:1 numerical non-disjunction are occasionally recovered. These of course can be recognized by the deviation from the normal chromosome number, though the different types possible cannot always be distinguished.

When the breaks occur near the middle of the arms, it becomes difficult to distinguish heterozygotes and deficiency-duplications. In the absence of phenotypic evidence from markers, the only (unsatisfactory) criterion may be recovery of certain plants with a considerably lower IV frequency than the heterozygote.

In addition to plants with one or more IV's, several lines studied had maximum associations of VI. Space does not permit a discussion here of the analysis of translocations giving hexavalents (which *a priori* may be due to either three or four breaks). Their behavior is very much more complex, inasmuch as 18, rather than 4, numerically balanced deficiency-duplications are possible upon outcrossing the heterozygote.

MATERIALS AND METHODS

In the fall of 1951, 60 seeds of a line (SL7-9) homozygous for five dominant marker genes² were soaked in tap water for 24 hours. The wet seeds with the root tips just beginning to emerge were exposed to 1200r of X-rays (measured with a Victoreen dosimeter), applied in four consecutive two-minute treatments of 300r each, at 160 KV and 15 ma. without filters. The X-ray machine (a Westinghouse 220 KV DuoCondex Deep Therapy Unit) was made available through the courtesy of the Department of Poultry Husbandry, Agricultural and Mechanical College of Texas. Fifty seeds with the strongest root tips were selected and planted two per greenhouse pot. Thirty-eight plants resulted. Pots with two plants were thinned to the strongest plant, leaving 25 experimental plants.

Acetocarmine smears of pollen mother cells from several plants were examined, and it was ascertained that the treatment had been effective in inducing chromosomal aberrations, and that some of the plants were chimeras for different chromosomal arrangements.

The 25 X_1 plants were crossed to various lines, all of which were recessive for the five markers of the X-rayed line, and some of which carried other dominant or recessive markers. One plant failed to set fruit, and one (Z1054) produced a single small boll containing only two seeds. The remaining 23 X_1 parents yielded one to several outcrossed bolls. All X_1 plants were discarded after the bolls were harvested. Individual bolls were harvested separately.

² R_1 = red body, R_2 = petal spot, L^0 = okra (dissected) leaf, K = brown lint, N = naked seed.

In the summer of 1953, progenies of about ten plants each from one to three bolls from each of the 23 fertile X_1 parents were planted in greenhouse cups and transplanted to field plots. Progenies from different bolls of the same parent were kept separate, as it was known that some of the parents were chimeras, and it was expected, and subsequently confirmed, that different bolls from the same parent would give different results. The two seeds from Z1054 were also planted, and the two plants were grown in the greenhouse. All progenies were scored for expression of the marker genes of the parents. Metaphase 1 configurations were studied in acetocarmine pollen mother cell smears from several plants in each progeny.

In addition, a preliminary study was made of chromosome aberrations induced by exposure of dry seeds to various dosages of thermal neutrons, and by growing plants under continuous Cobalt 60 irradiation. This material was provided through the courtesy of Dr. W. R. Singleton of the Brookhaven National Laboratory. Both types of treatment gave many interesting aberrations on which further study is planned. In general, the types of chromosome change were similar to those in the X-rayed material.

RESULTS OF THE X-RAY EXPERIMENT

The first outcross generation from the X_1 parents consisted of 47 progenies from 24 parents. Cytological aberrations were present in 17 progenies from 12 parents. Of the parents which gave aberrations, three gave more than one progeny with aberrations, five gave one progeny with aberrations and one or more with only normal plants, and four were represented by plants from only one boll. Two of the aberrant progenies, from two different parents, contained only monosomic and normal plants and will not be considered further here. The remaining 15 progenies had some plants with associations of more than two chromosomes, indicating the presence of segmental interchanges.

When interchanges were found in more than one progeny from a parent, it was not possible to decide with certainty in this generation whether the same or different interchanges were involved, because of the chimeral nature of some of the X_1 parents. Therefore in the analysis to follow, only the progeny from each parent from which the most data were obtained will be considered, with one exception. Parent Z1043 gave three progenies, all with a single IV and all showing the same cytological features. These three will be considered as a single progeny of 29 plants.

It is further assumed that, although different bolls from the same plant often gave different results, single bolls were not chimeral in origin, and that differences between individual plants were due to segregation, and not to their having descended from tissues of different chromosomal structure. No data were obtained which refuted this assumption, and even should it prove to be in error in individual cases, the general conclusions would not be greatly affected.

These considerations narrow discussion to ten lines in which translocations may be considered to have been induced independently of each other.

TABLE 1
RESULTS OF CYTOLOGICAL ANALYSIS OF TEN LINES CARRYING
X-RAY-INDUCED TRANSLOCATIONS

Parent (line)	Progeny No.	Max. assoc.	Min. No. breaks	Analyzed Plants			Type of het. (Fig. 1)
				Normal	Het.	Def.-dupl. No. Kinds	
Z1036	116D	IV	2	5	1? 1?	aIII
Z1039	118C	VI	3	2	3 2+
Z1040	119B*	VI	3	7 3
Z1043	121A-C	IV	2	9	7	10 1-2	aI
Z1047	122D	IV + III**	4 (IV)	2	4	2 1	bII
			(III)	2	6 1	(aI)
Z1048	123C	IV	2	1	2	3 1+	aIII
Z1052	125C*	IV	2	2	4 1†
Z1054	greenhouse	VI	3	2 2
Z1058	127B*	2IV	4 (IVa)	4	1	2 1	aIII
			(IVb)	2	4	1? 1?	aI
Z1059	127C	IV	2	5	3 2	aIII
			29				

*Presence of deficiency-duplications supported by genetic evidence.

**Assumed to have arisen from a potential IV.

†A second type of phenotypically detected deficiency-duplication was not analyzed cytologically.

These ten lines had twelve translocation complexes, the pertinent features of which are summarized in table 1. Eight of the complexes gave IV's, while another, which gave only a III (one centromere missing) is considered to have arisen from a potential IV. Three gave VI's, and must have involved translocation of at least three ends. A minimum of 29 translocated ends was involved in the 12 complexes. Since *G. hirsutum* has a haploid number of 26, some chromosomes must have been represented by more than one rearrangement.

Evidence of deficiency-duplications was obtained in all three of the lines with VI's. In one line (from Z1054), only two plants were recovered, one having 23 II 1 V (one chromosome missing), and the other usually an open IV, but rarely a VI. In Z1039, two plants (the heterozygotes) had ring VI's, one formed a V + I, and the rest only IV's. In the third (from Z1040), the heterozygote was not recovered, but the three types of deficiency-duplications were accompanied by genetic segregation which confirmed the cytological interpretation (see below).

In five of the lines with maximum complexes of IV, evidence for deficiency-duplication was entirely cytological:

Z1036—Five plants had ring IV's. In a sixth plant, only open IV's were seen, but since only nine cells were examined, its genotype was questionable.

Z1043—Seven plants had ring IV's. In 104 cells from these plants, a IV was present in 57 per cent of the cells, and 64 per cent of the IV's were

rings. Ten plants never formed rings, and in 354 cells from these plants, a IV was present in only 27 per cent of the cells.

Z1047—This line had two complexes. One was represented by two II's in three plants, and by a large III (one centromere missing) in five plants. For the other complex, an unequal translocation between two small chromosomes, two plants were normal, four were heterozygous, and two had an unequal II with a chiasma in only one arm (deficient for the long, and duplicate for the short, translocated arm).

Z1048—Three plants formed ring IV's. In 61 cells, a IV was present 93 per cent of the time, and 84 per cent of the IV's were rings. Three plants with IV's never formed rings. In 109 cells from these plants, only 40 per cent had a IV. In the plants with rings, only one out of 57 IV's had an interstitial chiasma, but in the plants which did not form rings, 15 out of 44 IV's had one interstitial chiasma.

Z1059—Five plants formed rings composed of one large, two medium-sized, and one small chromosome. A IV was present in all but three of 103 pollen mother cells, and 68 per cent of the IV's were rings. Four plants did not form rings. In three of these, the IV was composed of three medium-sized and one large chromosome, and a IV was present in 45 per cent of 65 cells examined. In the fourth plant, the most frequent configuration was a III + I (in eight out of 17 cells), and it was considered to be a deficiency-duplication different from the first three.

In three of the lines (Z1040, Z1052, and Z1058), the phenotypic expression of one or more of the marker genes of the X_1 parent supported the cytological evidence for the recovery of deficiency-duplications (table 2). The genes L° , K and N are semidominant, so that the heterozygote and homozygotes can be distinguished under field conditions. R_2R_2 and R_2r_2 are not distinguishable, but the absence of R_2 can be detected by the absence of petal spot in the lines under consideration here. R_1R_1 and R_1r_1 can be distinguished in most segregating populations, but in the present study, this distinction is qualified by the fact that damaged or dwarfed plants heterozygous for R_1 sometimes show an intensification of anthocyanin which shifts the phenotype toward that of R_1R_1 .

In line Z1040, only one plant (which died before it could be analyzed) had the expected $L^\circ 1$ phenotype. Six plants were phenotypically like $L^\circ L^\circ$, and three like 11. Since the X_1 parent was $L^\circ L^\circ$ and the outcross parent 11, these phenotypes are interpreted as respectively duplicate ($L^\circ L^\circ/1$) and deficient ($-/1$) for the chromosome segment carrying L° from the X_1 parent. Similar reasoning applies to the recovery of dark red, broad-leaved plants from Z1058, that is, they are probably R_1R_1/r_1 and $-/1$, and to plants with dark brown (KK/k) and white ($-/k$) lint from Z1052.

Z1052 gave four plants with dark brown lint, all of which were light (heterozygous) red and had petal spot. Another plant had white lint, no petal spot ($-/r_2$) and was dark red. Unfortunately this plant was small and unthrifty, and died before an analysis could be obtained. No evidence for more than one IV was seen in any of the plants analyzed. Since R_1 , R_2 and

TABLE 2

CYTOLOGICAL AND GENETIC CHARACTERISTICS OF THREE X-RAY-INDUCED LINES WHICH SHOWED ABERRATIONS FOR MARKER GENES OF THE X_1 PARENT

X_1 parent (line)	Phenotype						No. plants	Cytological configuration
	R_1	R_2^*	L°	K	N	Other		
Z1040	het	het	het	het	het	med. seed	1	not analyzed
	het	het	$L^\circ L^\circ/1$	het	het	small seed	6	4=open IV
	het	het	-/1	het	het	large seed	3	1=VI; 1=24 II, 1 III
Z1052	het	het	het	het	het	leaf not ruffled	4	2=26 II
	het	(het)**	het	KK/k	het	leaf margin ruffled	5	4=IV (not a ring)
	$R_1R_1/r_1?$	-/r	het	-/k	het	leaf not ruffled	1	not analyzed
Z1058	het	het	het	het	het	7	2=26 II; 1=2 IV, both rings; 1=1 IV; ring; 1=2 IV, 1 ring, 1 open.
	R_1R_1/r_1	het	-/1	het	het	2	2=2 IV, 1 ring, 1 always open

* R_2r_2 and R_2R_2 cannot be distinguished with certainty. Plants were scored as heterozygous if they had petal spot.

**It is suspected (see text) that these plants may have been R_2R_2/r_2 . Two plants when transplanted to the greenhouse proved to have more intense petal spots than other plants known to be heterozygous.

K segregate independently (C. F. Lewis, unpublished), one explanation of the results is that the dwarf plant was a deficiency-duplication, $-/r_2$ and R_1R_1/r_1 , from a second translocation, but that it was of the cIII type, figure 1 (R_1 and R_2 are known to be located in different genomes), and was not detected cytologically.

However, Rhyné (1954) has obtained evidence which suggests that R_2 and K may actually be located on the same chromosome, even though they show independent assortment. If this were the case, the KK/k plants might also have been R_2R_2/r_2 , phenotypically undetected, and the white linted plants, $-/r_2$ $-/k$. The dark red coloration may have been due to dwarf intensification of heterozygous R_1 , rather than to duplication for R_1 . In support of this second hypothesis, it may be noted that at least two of the KK/k plants were observed to have more intense petal spots than other heterozygotes after transplanting to the greenhouse in the fall.

In two progenies, the aberrant behavior of the SL7-9 markers was correlated with another phenotypic character (table 2). In Z1052, KK/k plants had ruffled leaf margins, while Kk and $-/k$ had non-ruffled leaves. In Z1040, $L^\circ L^\circ/1$ plants had small seeds, $-/1$ plants had large seeds, and the single $L^\circ 1$ plants had seeds of intermediate size (about the size of normal SL7-9 heterozygotes). Seed size was not associated with a difference in germination. The significance of these two characteristics remains to be determined.

Cytological data agreed with the interpretation of the genetically aberrant plants as deficiency-duplications (table 2). All genetic aberrations were

accompanied by translocation figures at metaphase I, which makes it seem unlikely that mutations accounted for any of them. All plants were heterozygous as expected for the other markers, so that contamination could be ruled out. Also, from two of the three parents concerned, progenies from other bolls were grown, and were both phenotypically and cytologically normal.

Consideration of the cytological data indicated that the markers which segregated in these lines were all located distal to the respective points of breakage, that is, the cytological configurations indicated deficiency-duplication for the translocated ends rather than the centromere regions and unbroken arms. One $-/1$ plant from Z1040 was also deficient for a centromere region, but the other $-/1$ plant, which had a VI, showed that this segment was not the one which carried L^o . Centromere deficiencies were recovered from two other lines (Z1047 and Z1054) without effect on the marker phenotype.

DISCUSSION

Even in the small progenies studied, definite cytological evidence, supported in three cases by genetic evidence, was obtained for the recovery of deficiency-duplications from 10 of the 12 complexes. Probably at least one deficiency-duplication was obtained from each of the other two. The number of plants available for analysis in this generation was too small to allow any significance to be placed on the frequencies of the various types in a progeny. But no normal plants were recovered from five complexes, no heterozygotes from four, and *only* deficiency-duplications from two of the VI's. The conclusion seems warranted that viable deficiency-duplications are the rule rather than the exception in translocations in *G. hirsutum*. This appears to hold true also for the naturally occurring translocations which have arisen during the divergence of the A_1 , A_2 and A_h genomes (Gerstel, 1953; Menzel and Brown, 1954; Menzel, in press).

Furthermore, none of the heterozygotes or deficiency-duplications was sterile, though some were less fertile than normal plants in the same progeny. Since *G. hirsutum* is an amphidiploid species, this high tolerance to alterations in chromosome balance is perhaps not surprising (Smith, 1948; Menzel and Brown, 1952). It suggests that deficiencies in one genome are often partially compensated by chromosome segments in the other genome, even though *G. hirsutum* behaves cytologically like a diploid in that it normally forms only II's at metaphase I.

Seven of the nine IV complexes could be assigned fairly easily to three of the ten types shown in figure 1. Three were aI, four were aIII, and one was bII. The heterozygote was not recovered for the other two complexes. While only three of the ten types were represented in this material, some of the other types have been recognized in other material. Brown (1949) found small rings or chains (aII) in siblings of 2B-1, as well as complexes of types aI and aIII. A spontaneous translocation (Z121, Brown, unpublished), which has been shown to involve two chromosomes different from

those involved in 2B-1, is similar to bI. Complexes corresponding to cI and cIII, and probably also to cII, have been found in the neutron and cobalt 60 treatments, in addition to types aI, aII, aIII, bI and possibly others. A number of the latter lines showed a high variability in the types of configurations formed, which is taken to indicate that the breaks occurred near the middle of the arms, rather than near the centromere or near the end.

While this evidence indicates that breaks may be induced anywhere along a chromosome arm, it cannot be said at present whether the breaks are randomly distributed, or whether they tend to occur most frequently in certain regions. If the type and frequency of configurations is a reliable index of the length of the arm involved, there is a suggestion in these preliminary data that breaks occur more frequently near the centromere, since the commonest maximum configuration encountered is a ring of IV rather than the configurations shown in figure 1, b and c.

Several lines have modal pairing of a chain of four, with only a few cells showing one or more of the configurations shown in figure 1. And at least two plants have been found in the T₁ generations of neutron-treated material (presumably heterozygous), in which the "rare" configuration of type c, a six-chiasma IV, is formed in almost every cell. In further studies, the working model presented in figure 1 may have to be altered if (1) chiasma frequency varies more than has been assumed from arm to arm in the complement, and (2) chiasmata tend to be localized in some or all arms, rather than distributed more or less at random throughout most of the length of the arm.

SUMMARY

Exposure of germinating seeds of *G. hirsutum* to 1200r of X-rays was effective in producing chromosome aberrations. Ten lines having 12 different translocation complexes were recovered, involving a minimum of 29 breaks and rearrangements. At least ten, and probably all twelve, of these complexes gave one or more viable and fertile deficiency-duplication genotypes when outcrossed. Several of the complexes differed in the size of the chromosomes and length of the interchanged arms in such a way as to permit identification of the heterozygote and some or all of the deficiency-duplications by the configurations formed at metaphase I. In three lines, deficiency-duplications for segments carrying marker genes were recovered.

It is concluded that the recovery of viable, fertile deficiency-duplications from translocations is the rule rather than the exception in this amphidiploid species.

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POSITION EFFECTS IN NATURAL POPULATIONS

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About thirty years ago Sturtevant (1925) discovered the phenomenon of position effects. Since then an extensive literature of laboratory studies of the phenomenon has developed (see reviews and discussions by Dobzhansky (1936), White (1941), and Lewis (1950)). However, no demonstrations of position effects in natural populations have been given. The data reported here show that position effects occur in natural populations of the fly *Drosophila robusta*.

MATERIALS AND METHODS

Adult flies of this species were collected over a period of four years in Crumpacker and Heth's Woods, located about two miles apart, near Blacksburg, Virginia. The methods used to collect the flies and to analyze their chromosome constitution were similar to those previously described by the author (Levitan, 1951a and b), except that for the last three years of the study inseminated females were also completely determined for gene arrangement (by despermizing, crossing to laboratory males, and studying salivary smears from at least eight of their progeny).

RESULTS

292 of the adults whose gene arrangements were completely determined for the V-shaped second chromosome proved to be heterozygous for the two common gene arrangements of the right arm, 2R and 2R-1. For detailed descriptions of 2R and 2R-1, which differ from one another by a terminal inversion, and of the other gene arrangements mentioned in this paper, the reader is referred to the work of Carson and Stalker (1947). The four arrangements of the left arm present in these populations are sometimes linked to 2R and sometimes to 2R-1. The number of each of these linkages found in the 2R/2R-1 individuals is shown in table 1. The linkages in six females heterozygous for arrangements in both arms could not be determined because recombination between the right- and left-arm arrangements was not significantly different from 50 per cent. The twelve chromosomes involved have been placed in the table as follows: one 2L 2R, one 2L 2R-1, two 2L-1 2R, and two 2L-2 2R-1 from Crumpacker Woods; two 2L 2R, one 2L-1 2R, and three 2L-3 2R-1 from Heth's Woods. This disposition is designed to minimize the main conclusions of the paper, but the effect of reversing the doubtful linkages will also be indicated.

If the arrangements of the left arm were independent of the ones on the right arm, the number of second chromosomes containing a given left-arm

TABLE 1
SECOND CHROMOSOMES PRESENT IN 292 2R/2R-1 INDIVIDUALS

Group	Chromosome	Crumpacker Woods			Heth's Woods			Both Woods		
		Females	Males	Total	Females	Males	Total	Females	Males	Total
1	2L 2R	18	26	44	19	10	29	37	36	73
	2L 2R-1	25	43*	68*	9	19	28	34	62**	96†
2	2L-1 2R	13	21	34	11	12	23	24	33	57
	2L-1 2R-1	14	35	49#	16	22	38#	30	57*	87*†
3	2L-2 2R	18	18	36	6	10	16	24	28	52
	2L-2 2R-1	17	13	30	7	8	15	24	21	45
4	2L-3 2R	24	44	68	12	30	42	36	74	110
	2L-3 2R-1	17	18***	35**	16	13**	29#	33	31****	64***†
Total		146	218	364	96	124	220	242	342	584

*Deviation from 1:1 ratio significant at the 5 per cent level.

**Deviation from 1:1 ratio significant at the 1 per cent level.

***Deviation from 1:1 ratio significant at the 0.1 per cent level.

****Deviation from 1:1 ratio significant at the 0.01 per cent level.

#Deviation from 1:1 ratio would be significant at the 5 per cent level if doubtful cases (see text) were reversed.

†Deviation from 1:1 ratio would be significant at a higher level (i.e., P would be lower) if doubtful cases were reversed.

TABLE 2
SUMS OF THE CHI-SQUARES TESTING DEVIATIONS FROM EQUALITY OF PAIRS IN TABLE 1.†

Table 1 groups included	d.f. of each sum	Crumpacker Woods			Heth's Woods			Both Woods		
		Females	Males	Total	Females	Males	Total	Females	Males	Total
1-4	4	2.4	19.4***	19.0***	5.1	12.7*	6.1#	0.9	31.9****	22.0***†
1-3	3	1.2	8.5*	8.4*	4.6	6.0	3.7	0.8	14.3**	9.9*†

†For explanation of symbols refer to bottom of table 1.

arrangement linked to 2R should be equal, in 2R/2R-1 individuals, to the number with the same arrangement linked to 2R-1. Table 1 shows that this is generally not so. Chi-squares testing deviation from a 1:1 ratio for each of the 36 pairs of data disclose ten significant at the 5 per cent, six at the 1 per cent level. Four others would be significant if the linkages of the doubtful females were reversed. When the chi-squares related to a given sample ("Crumpacker Woods males", etc.) are added (table 2), five of the nine are significant at, at least, the 5 per cent level. Removing the large chi-squares related to chromosomes with arrangement 2L-3 leaves four of the nine samples significant (row 2 of table 2). Similarly, samples combining data from the two woods tend to have higher degrees of significance than the material from a single one, because the nonsignificant deviations from equality are often in the same direction as the significant ones. This is particularly noticeable in the male data, where the deviations are generally large even when they are not significant. Thus, ten of the twelve chi-squares for "pairs" in the male data have P less than 10 per cent, as do five of the six chi-squares for "samples." In the corresponding female data only one of the twelve pairs and none of the six samples have P below 10 per cent. Interestingly enough, both the male pairs with P above 10 per cent concern 2L-2, which has no significant deviations in any of the data, and the lone female P below 10 per cent depends on the way the chromosomes from the doubtful females are counted.

DISCUSSION

The differences between the male and female data eliminate one of the possible explanations for the non-random associations between left- and right-arm arrangements, namely, that it is a relic of the mode of origin of the arrangements. On this hypothesis, the larger number of 2L-3 chromosomes attached to 2R than to 2R-1 would be attributable to 2R-1's having arisen on a chromosome which had 2L or 2L-1 in the left arm, in an area where 2L-3 was rare or absent (or, vice versa, to 2L-3's having arisen on a chromosome which also carried 2R, in an area where 2R-1 was rare). Similarly, 2L and 2L-1 would be visualized as having been attached to 2R-1 long before they encountered 2R. Even had these inversions originated as stated, however, the female data show that recombination between the right- and left-arm arrangements has occurred often enough since then to allow the linkages in 2R/2R-1 individuals to be uniform. Theoretical considerations (e.g., Geiringer, 1944, 1948) also lead to the conclusion that these linked arrangements should be found in "populational independence," inasmuch as recombination between them is possible (Carson, 1953; Levitan, 1953a, b, and unpublished data), it is probably equal in coupling and repulsion phases, and the arrangements have probably coexisted in this area for a long time (Carson and Stalker, 1947)—the point being that recombination has probably been occurring for a long time. Consequently, significant deviation from independence, as observed here in the males, must be the result of selec-

tion. It appears to be another instance of the selectional differences between the two sexes noted earlier in this species (Levitan, 1951b).

Attributing this selection to any right-arm arrangement alone or to any left-arm arrangement alone meets with contradictions in the data. It might be supposed then that the data result from selection for individuals which have both a certain left-arm arrangement and a certain right-arm arrangement, because the interaction of genes trapped in the two arrangements produces a favorable phenotype. This hypothesis is also inadequate. Under it the pertinent genes need not be on the same chromosome; they could be on different homologous chromosomes to give the same interaction. As an example it could be assumed that simultaneous heterozygosity for loci A, B, C, and D forms a selectionally advantageous phenotype, and that A and B are held together by the absence of recombination within arrangement 2L-3, a and b in 2L, C and D in 2R, and c and d in 2R-1. The appropriate phenotype could be formed as well in the linkage relationship $ABcd/abCD$ (2L 2R/2L-3 2R-1 in terms of the inversions) as in the relationship $ABCD/abcd$ (2L 2R-1/2L-3 2R). Hence, there would be no need for inequalities as between, say, $2L-3$ 2R and $2L-3$ 2R-1 in 2R/2R-1 individuals. The only satisfactory explanation for the observed inequalities is that the pertinent genes on the two arms must be in a specific positional relationship to produce the phenotype in question. Using the above example with reference to table 1, this would mean that the phenotype produced by having ABCD on one homologue and abcd on the other was different (and selectionally more favorable) than the phenotype produced by having the same genes in different position in the cell, ABcd on one homologue and abCD on the other. The crucial point in the formation of these phenotypes is, therefore, a position effect.

A more detailed description of these results and of their implications is being prepared for publication in another journal.

SUMMARY

Analysis of adult *Drosophila robusta* collected near Blacksburg, Virginia, discloses non-random associations between gene arrangements of the left and right arms of the V-shaped second chromosome, particularly in the males. Consideration of various hypotheses to account for these results leads to the conclusion that selection for position effects is responsible for them.

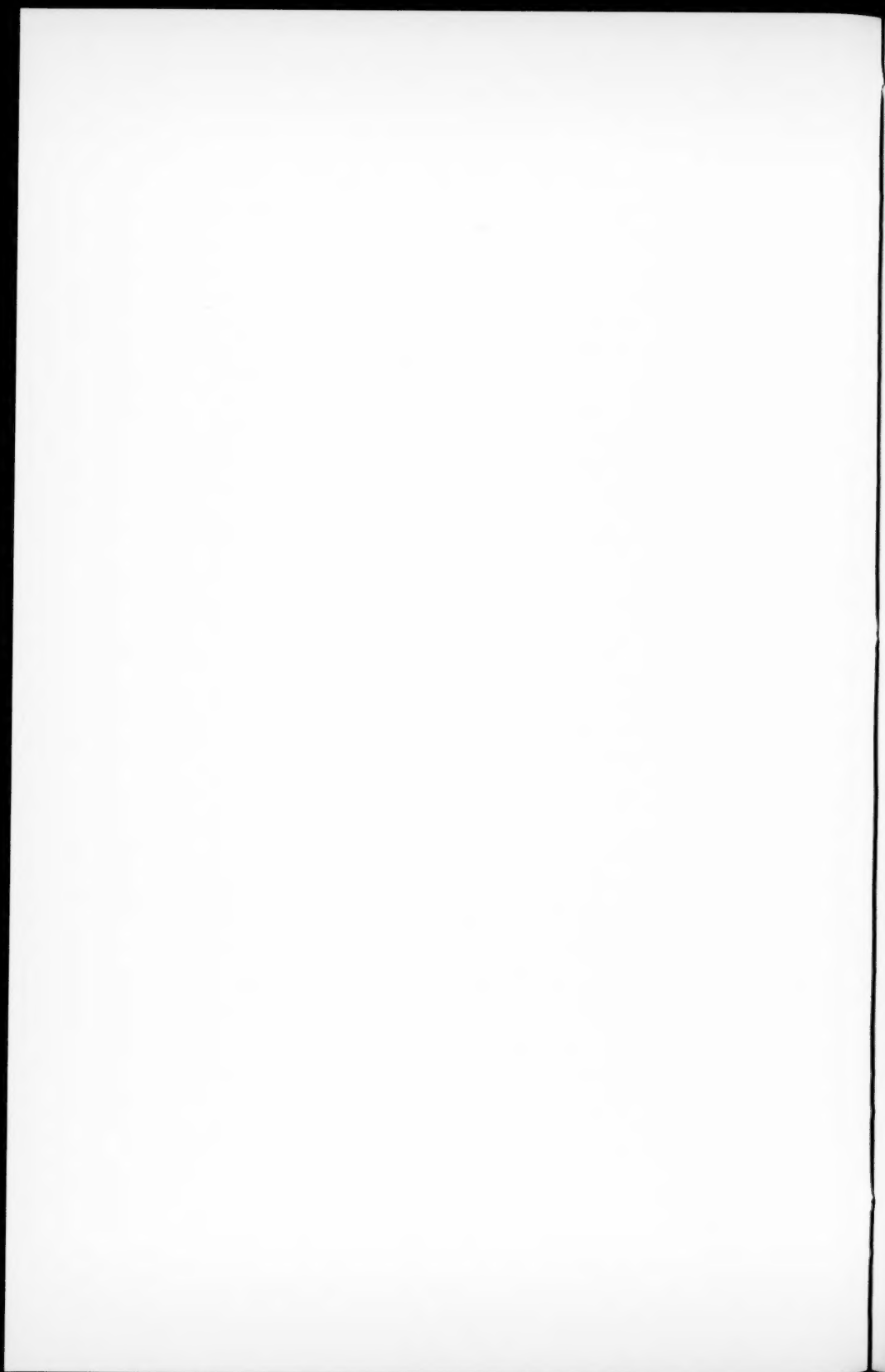
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Note added in proof: One 2L-2R-1 and one 2L-2-2R from a Crumppacker male were accidentally omitted from Table 1. These increase slightly the numbers in columns 2, 3, 8, and 9 of Table 2, but they make no changes in the number of significant results.

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RADIATION-INDUCED PYCNOSIS OF CHROMOSOMES AND
ITS RELATION TO OXYGEN TENSION¹

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Although breakage of chromosomes, together with the recombination of broken ends, constitutes the most conspicuous and most readily analyzable of the cytological consequences of the exposure of cells to ionizing radiations, the pycnotic effects of radiations have long been recognized (see Darlington and La Cour, 1945, for earlier references). These effects, defined as the sticking of chromatids during anaphase separation and by the clumping of chromosomes at metaphase, have usually proved to be a hindrance to the accurate analysis of induced aberrations when both effects are present, but since the former usually precedes breakage in time, this difficulty is easily avoided. Despite the great amount of quantitative information available regarding the relation of frequency of aberrations to dosage, there is a relative dearth of similar data pertaining to the pycnotic effects. Darlington and La Cour (1945) have reported some findings, as have Koller (1953) and Thoday and Read (1949), but the need for additional information is apparent when it is considered that there is a possible relation between stickiness and other cellular effects of radiation which has not been resolved. For example, the "sticky" gene in maize is known to cause, when in a homozygous condition, both stickiness of chromosomes as well as an increased frequency of aberrations and mutations. To be sure, the "sticky" phenomenon in maize may well be a primary effect, with breakage and mutation increases secondary, but there is no certainty of this; both may, in fact, stem from the same basic instability yet be independent of each other. It seemed desirable, therefore, to test in *Tradescantia* microspore cells the effects of a reduced oxygen tension during X irradiation on the degree of expression of pycnosis, and further to determine, if a reduction of effect is achieved, whether this reduction bears any quantitative relation to the reduction found for frequencies of aberrations.

Figure 1 presents in graphic form the data which were obtained. Each point on the curves is based on 200-300 observations. Scoring of cells was confined to clumped metaphases, although enough information was obtained to show that anaphase stickiness was evident sooner after radiation, affected a greater proportion of cells, and disappeared later.

Figure 1 shows that after exposure of cells to 150 r in air, stickiness is not immediately evident but reaches full expression only after the passage of an hour's time (curve A). All metaphases remain clumped until about 9

¹This work was supported by USAEC Contracts No. W-7405-eng-26 and No. AT-(30-1)-851.

hours after irradiation, at which time recovery begins and continues until no clumping is found at 12 hours. In the nitrogen series, 150 r produced no visible clumping of metaphases, and a dose of 600 r was required to achieve approximately the same severity and duration of clumping as that induced by 150 r in air. The air/nitrogen ratio for pycnosis, therefore, is roughly 4 for the 1-to-9-hour period after irradiation. Lower doses of radiation in air and in nitrogen gave reduced effect, as indicated in figure 1 (curves B and C). These lower doses led to a later appearance of clumping, an earlier disappearance, and a lower proportion of affected cells.

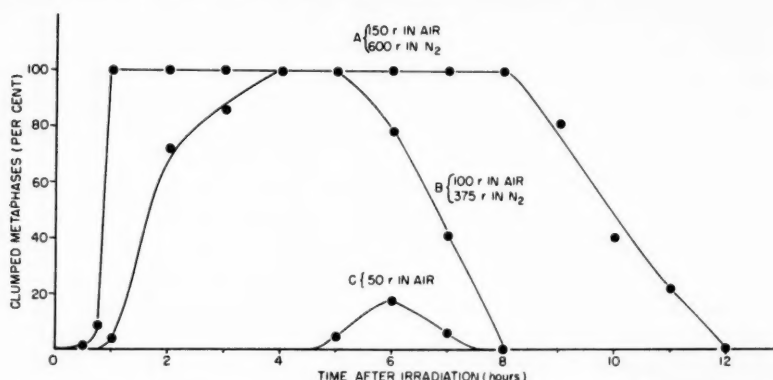


FIGURE 1. Relation of pycnosis as evidenced through clumped metaphases to time after irradiation.

These data were obtained during the winter months when the division cycle in *Tradescantia* proceeds at a much slower tempo than during the warmer months. Additional studies carried out during the spring and summer confirmed in general the earlier data given in figure 1, but indicated further that the duration of the "sticky" period is related to the rapidity of cell division, i.e., the more rapid the cell division the more rapid is the initiation of, as well as the recovery of cells from, radiation-induced pycnosis. These findings are in essential agreement with those of Darlington and La Cour (1945).

The results presented here indicate that the reduction in frequency of chromosomal aberrations, obtained when cells in an atmosphere of lower oxygen tension are exposed to ionizing radiations (e.g., see Giles, 1952), is paralleled by a reduction in pycnosis. The air/nitrogen ratios for both effects are roughly comparable in magnitude. The parallelism, however, extends beyond these facts and raises three questions, answers to which seem particularly pertinent to an understanding of radiobiological effects at the cellular level. These are: What is the nature of pycnosis, and how does a reduced oxygen tension alter its expression? What is the relation between frequency of aberrations and pycnosis? Is pycnosis related in any way to radiation-induced retardation of cell division? No positive answers can be

given at this time, but it seems worth while to point out certain facts which may or may not be causally related.

As to the nature of the pycnotic effect, Darlington and La Cour (1945) have expressed the opinion that X-rays overcharge the chromosomes with DNA and that the DNA of the chromosome is in a fluid and depolymerized state, and hence "sticky." These investigators further state that stickiness is "a term which is now resolving itself into meaning errors of reproduction" (Darlington and La Cour, 1953). It should be recognized that these are operational descriptions of observed events, and that other interpretations are possible. *In vitro* studies of X irradiated DNA solutions, however, have a possible bearing on these interpretations and findings. Taylor, Greenstein, and Hollaender (1947, 1948) have shown that solutions of irradiated DNA continue to depolymerize for long periods of time after cessation of the irradiation, supporting, to a certain extent at least, the contention that stickiness in chromosomes is due to depolymerized DNA. It may well be, as Butler and Smith (1950) have demonstrated, that short-lived free radicals can initiate a chain reaction which, by means of intermediate products, continues the degradation of DNA. Degradation, as shown by a decrease in viscosity, ceases, however, when irradiation ceases, provided that the solution of DNA has been prepared and irradiated under oxygen-free conditions (Butler and Conway, 1950). Degradation of DNA when oxygen is present and pycnosis of chromosomes, if extension of this *in vitro* observation is made to the present findings, seems therefore to be due to a product of molecular oxygen, which Butler and Conway believe cannot be hydrogen peroxide since none was detected, although organic peroxides appear not to be eliminated as mediating substances. Further extrapolation of the findings from *in vitro* studies to the *Tradescantia* data, however suggestive the comparative aspects may be, becomes risky until a more accurate comparison can be made of the curves obtained by plotting pycnosis and DNA viscosity as a function of time after irradiation. Cytochemical studies on irradiated chromosomes are also needed before it can be said with certainty that pycnosis is actually due to degraded DNA.

There remains another study the results of which have many points in common with the progressive change in DNA which, after cessation of irradiation, probably leads to pycnosis in the present case and to continued degradation *in vitro*. This is the study by Alper (quoted by Gray, 1953), who has shown that inactivation of phage particles by X-rays is not only oxygen sensitive, but also that the degree of inactivation increases as the time between irradiation and plating increases. The progressive inactivation has been traced to an interaction which takes place between the injured phage and H_2O_2 . The injured phage, production of which is oxygen independent, is capable of further change toward lethality in the presence of oxygen, or is capable of a return to an uninjured state in the absence of oxygen, or through plating which ties up and makes H_2O_2 ineffective. If pycnosis and phage injury are thought of as having a basis in an altered

DNA, the parallel is striking indeed, even though the speculative nature of the comparison should be recognized.

Furthermore, if the oxygen-sensitive aspects are considered to be the "indirect" effects of radiation (Giles, 1952), at the same time keeping in mind that the influence of oxygen on radiosensitivity (i.e., production of aberrations) is very closely linked in an inverse relation to the type of radiation employed—being greatest with X-rays, less with neutrons, and still less with alpha rays—it would be expected that of the total effect of any radiation in air a greater proportion of the effect would be indirect the less densely ionizing the radiation. Inactivation of phage particles has not yet, to our knowledge, been investigated from this point of view, but support is provided by Koller (1953) who has indicated that pycnosis is often greater with gamma rays than with X-rays, and by Thoday and Read (1949) who have stated that it is more pronounced with X-rays than with alpha rays. It can be considered therefore that pycnosis is an indirect effect, that it is oxygen sensitive as to its expression, and that it is very likely due to a progressive change in DNA which results from the action of active radicals generated in the cell by radiation. Whether the suggestion of Darlington and La Cour (1953) that stickiness is an "error in reproduction" is accepted will depend on how we wish to define reproduction.

A dependent rather than a merely coincidental relation of radiation-induced pycnosis to the production of aberrations is indicated, although by no means proved, by several studies. The action of the recessive gene "sticky" in maize has already been mentioned. The comparable air/nitrogen ratios for pycnosis reported in this paper, and those reported for chromosomal aberrations point in this direction, as do the comparative studies made with different kinds of radiation (Thoday and Read, 1949; Giles, Beatty, and Riley, 1952). It should, however, be made clear that pycnosis is visible only in late prophase, metaphase, or anaphase stages of cell division, whereas breakage, healing, restitution, and recombination, as commonly investigated, take place in early prophase (chromatid aberrations) or resting stage (chromosomal aberrations) nuclei. On the other hand, if there are DNA changes in early prophase and resting stage nuclei which cannot be detected microscopically but which are comparable to those visible in later stages, it may well be that the frequency of detectable aberrations is partially governed by these changes. Certain experiments can be done to test this hypothesis. For example, if it is assumed that the effect of a reduced oxygen tension is on restitution and recombination rather than on breakage, or, alternatively, on the probability of realization of primary breaks from potential breaks (Thoday, 1953; Swanson, *in press*), then logically it might be expected that a fractionation experiment, with one dose in air and another in nitrogen given to the same set of cells, would show that an air-nitrogen treatment would be more effective in inducing aberrations than a nitrogen-air treatment for the reason that the pycnosis induced by the original exposure in air would make more effective the subsequent exposure in nitrogen.

On the other hand, if the initial exposure in nitrogen induced no pycnosis, a subsequent exposure in air would be simply additive rather than synergistic, provided, of course, that the two fractions of radiation were sufficiently separated in time to prevent the interaction of the two sets of broken ends. It is realized that these predictions are based on an opposite point of view from that expressed by Lane (1951, 1953) who holds that the sensitivity of chromosomes is reduced by one dose in such a way that a second and equal dose is less effective, but preliminary experiments, done in these laboratories and using doses of 100 r in air and the same dose in nitrogen, strongly suggest that the expected situation is realized in that the air-nitrogen exposure is more effective than the nitrogen-air combination. The need for extensive confirmation, however, urges caution before these tentative results can be accepted for support of the hypothesis. The need for caution is further emphasized by the studies on chemical mutagenesis. D'Amato (1952) believes that "stickiness and chromosome breakage are not interdependent and necessarily connected events in the mutagenic activity of a chemical compound," a belief arising from the observations that some chemicals such as ethylene glycol and ethyl alcohol induce pycnosis without aberrations, whereas maleic hydrazide and some acridines and purines induce aberrations but no pycnosis. Many chemicals are known which induce both effects, but the difficulty lies in equating the actions of X-rays and chemicals on chromosomes. The point of view presented here, that pycnosis may govern in part the processes which follow initial damage by radiation, is not incompatible with D'Amato's belief of the independence of the two effects, but merely suggests that the same frequency of initial effects will yield a higher frequency of detectable aberrations if pycnosis intervenes.

As to the relation between radiation-induced pycnosis and mitotic delay, mention can be made of Gaulden's observations (Gaulden, Nix, and Moshman, 1953) which showed that grasshopper neuroblast cells irradiated in nitrogen recovered their rate of division more rapidly than similar cells similarly exposed in air. Mitotic inhibition and the frequency of chromosomal fragmentation, both of which vary systematically with the stage of division, are also correlated in Trillium (Sparrow, Moses, and DuBow, 1952). These are perhaps more tenuous parallelisms than those discussed, but it is through the pursuit of such correlative relation that possible avenues are opened up to an understanding of cellular damage by radiations.

SUMMARY

It has been shown that the pycnotic effect of radiation is markedly reduced when exposure of cells is made in nitrogen as opposed to air. A discussion of the possible relations of induced pycnosis to DNA studies *in vitro*, to aberration production, and briefly to mitotic delay indicates that a study of these effects, more or less avoided in the past, may advance knowledge of radiation biology.

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LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

RANDOM MATING WITH LINKAGE IN POLYSOMICS*

In a recent paper Bennett (1954)¹ has presented formulae for the approach to equilibrium of linked loci in a panmictic population of tetra- and hexasomics. Bennett's methods are rather advanced, but his equations can be obtained quite simply using only elementary methods. The method for tetrasomics is presented here since it is convenient for teaching and may possibly be useful for other problems.

We assume that crossovers are equally likely to occur between chromatids from any two of the four chromosomes, but the association of crossover and non-crossover chromatids in gametes need not be random and double reduction is permitted.

Let $p(A)$ and $p(B)$ be the frequencies of genes A and B. In the absence of selection and mutation these frequencies will not change. Let $p_n(AB)$ be the probability that a chromosome chosen at random from the gametes produced by the n th generation will carry genes A and B. Let y be the recombination fraction.

A chromosome chosen at random from a gamete produced by generation $n + 1$ has a probability $1 - y$ of not having been involved in a crossover, in which case it has the same probability of carrying genes A and B as in a gamete from the previous generation, i.e., $p_n(AB)$. It has a probability y of having been involved in a crossover. Two-thirds of the crossovers involve chromosomes that came from different gametes and, with random mating, the A locus in one chromosome and the B locus in the other are independent. Thus the probability that such a crossover chromosome carries A and B is $p(A)p(B)$. The remaining third of the crossovers involve chromosomes from the same gamete which could lead to an AB chromosome if one chromosome carried A and the other B. Let $p_n(A/B)$ be the probability that the chromosome chosen at random contains gene A and that the other chromosome in the same gamete contains B. Hence,

$$\begin{aligned} p_{n+1}(AB) &= (1 - y)p_n(AB) + y \left[\frac{2}{3} p(A)p(B) + \frac{1}{3} p_n(A/B) \right] \\ &= p_n(AB) + \frac{2y}{3} [p(A)p(B) - p_n(AB)] + \frac{y}{3} [p_n(A/B) - p_n(AB)] \quad (1) \end{aligned}$$

*Paper No. 556 from the Department of Genetics, University of Wisconsin, Madison, Wisconsin.

TABLE 1
PROBABILITY OF (AB) AND (A/B) CHROMOSOMES IN GAMETES ACCORDING TO VARIOUS MODES OF GAMETE FORMATION.
MODES OF GAMETE FORMATION AFTER FISHER, 1947, FROM A ZYGOTE OF GENOTYPE $a_1b_1/a_2b_2/a_3b_3/a_4b_4$

Mode of formation	Typical gamete	Probability of chromosome makeup in gamete	
		$P_{n+1}(AB)$	$P_{n+1}(A/B)$
1	a_1b_1/a_2b_2	$p_n(AB)$	$[2p(A)p(B) + p_n(A/B)]/3$
2	a_1b_1/a_1b_1	$p_n(AB)$	$p_n(AB)$
3	a_1b_1/a_2b_3	$[3p_n(AB) + 2p(A)p(B) + p_n(A/B)]/6$	$[2p(A)p(B) + p_n(A/B)]/3$
4	a_1b_1/a_1b_2	$[3p_n(AB) + 2p(A)p(B) + p_n(A/B)]/6$	$[3p_n(AB) + 2p(A)(B) + p_n(A/B)]/6$
5	a_1b_1/a_2b_1		
6	a_1b_2/a_2b_3	$[2p(A)p(B) + p_n(A/B)]/3$	$[2p(A)p(B) + p_n(A/B)]/3$
7	a_2b_1/a_3b_1		
8	a_1b_2/a_1b_1	$[2p(A)p(B) + p_n(A/B)]/3$	$[3p_n(AB) + 2p(A)p(B) + p(A/B)]/6$
9	a_1b_2/a_2b_3	$[2p(A)p(B) + p_n(A/B)]/3$	$[2p(A)p(B) + p_n(A/B)]/3$
10	a_1b_2/a_1b_2	$[2p(A)p(B) + p_n(A/B)]/3$	$p_n(AB)$
11	a_1b_2/a_2b_1		

The modes of gamete formation in a tetraploid with two loci are of eleven essentially different kinds as enumerated by Fisher (1947).² Table 1 gives for each mode of gamete formation the probability of AB and A/B chromosomes in gametes of generation $n + 1$ in terms of the corresponding probabilities in the n th generation. For example, with mode 1 (neither chromosome in the gamete involved in a crossover) $p_{n+1}(AB) = p_n(AB)$, since without crossovers the probability of an AB chromosome does not change in succeeding generations. Likewise, $p_{n+1}(A/B) = \frac{2}{3}p(A)p(B) + \frac{1}{3}p_n(A/B)$, since the two chromosomes in the gamete have a probability of $\frac{2}{3}$ of being from different parents (in which case they are independent) and $\frac{1}{3}$ of being from the same parent (in which case the probability is the same as for the previous generation).

In this manner all the probabilities in table 1 may be obtained, since each mode of gamete formation specifies the nature of the relevant crossovers which have occurred. The two probabilities are equal for most modes of gamete formation and for the remaining modes they differ by some multiple of $3p_n(AB) - 2p(A)p(B) - p_n(A/B)$. Therefore,

$$p_{n+1}(AB) - p_{n+1}(A/B) = \frac{k}{6} [3p_n(AB) - 2p(A)p(B) - p_n(A/B)] \quad (2)$$

where $k = 2(f_1 - f_{11}) + f_3 - f_9$

f_i = frequency of the i th mode of gamete formation

Equation (1) may be rewritten as

$$p_{n+1}(AB) - p_n(AB) = \frac{-y}{3} [3p_n(AB) - 2p(A)p(B) - p_n(A/B)]$$

Hence, from this and (2)

$$y [p_n(AB) - p_n(A/B)] = \frac{-k}{2} [p_n(AB) - p_{n-1}(AB)]$$

Substituting in (1), we obtain

$$p_{n+1}(AB) = p_n(AB) + \frac{2y}{3} [p(A)p(B) - p_n(AB)] + \frac{k}{6} [p_n(AB) - p_{n-1}(AB)] \quad (3)$$

This is the same as Bennett's (13). It may be conveniently written

$$z_{n+1} = Dz_n + Ez_{n-1} \quad (4)$$

where

$$z_n = p_n(AB) - p(A)p(B)$$

$$D = 1 + k/6 - 2y/3$$

$$E = -k/6$$

Equation (4) is a linear sequence equation of the second order and has a standard solution (see, for example, Hogben (1946), p. 50-55).⁴

The solution may be written

$$z_n = pR^n + qS^n \quad (5)$$

where
$$p = \frac{z_0 S - z_1}{S - R}$$

$$q = \frac{z_0 R - z_1}{R - S}$$

$$R = \frac{D + \sqrt{D^2 + 4E}}{2}$$

$$S = \frac{D - \sqrt{D^2 + 4E}}{2}$$

R and S are the same as the latent roots, λ and μ , of Bennett.

To find the equilibrium values, we note that when $y = 0$, $R = 1$; and for all other possible values of y , $|R| < 1$. If $|R| < 1$, then $|S| < 1$. Therefore as n becomes large, $z_n \rightarrow 0$; i.e., at equilibrium $p(AB) = p(A)p(B)$.

Thus the chromosome frequencies approach a state in which the joint probabilities are the products of the individual gene frequencies. On the other hand the equilibrium frequencies of zygotic types for a single polysomic locus are not given by expansion of the gene frequencies, except in the absence of double reduction (Geiringer, 1949).³

Equation (4) also holds for hexasomics, in which case $D = 1 + \frac{k'}{15} - \frac{3y}{15}$

and $E = -\frac{k'}{15}$ (Bennett, 1954), and the value of k' is given by Bennett's equation (19). In diploids, $D = 1 - y$ and $E = 0$, leading to the familiar formula that the departure from equilibrium is reduced each generation by a proportion equal to the recombination fraction.

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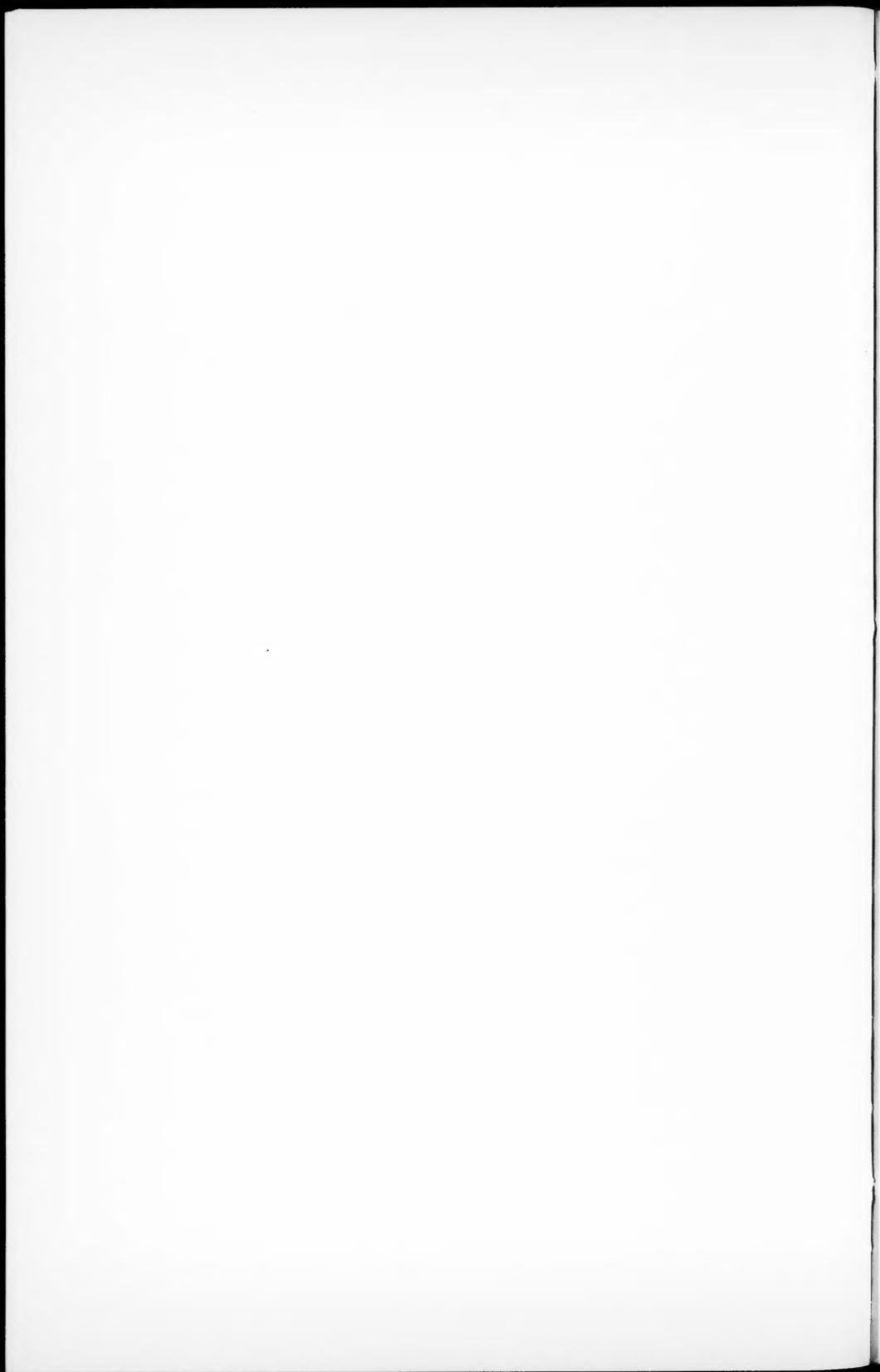
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THE RELATION OF NEUTRON DOSE TO CHROMOSOME
CHANGES AND POINT MUTATIONS IN DROSOPHILA.
I. TRANSLOCATIONS

H. J. Muller



THE RELATION OF NEUTRON DOSE TO CHROMOSOME CHANGES
AND POINT MUTATIONS IN *DROSOPHILA*.

I. TRANSLOCATIONS*

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1. NATURE OF THE PRESENT INVESTIGATION

The subject of the production of gene and chromosome changes by application of fast neutrons to *Drosophila* spermatozoa, first opened up by Nagai and Locher (1937, 1938) working under Altenburg's direction, is now due for radical overhauling, because of advances in dosimetry, in genetic techniques, and in understanding of both the physicochemical and the biological phenomena involved. Of the three series of experiments here to be reported, although two of them, done in 1949 to 1951 in collaboration with J. I. and R. M. Valencia, J. Kramer, F. Verderosa and E. Roeper, have already had certain of their findings briefly discussed (Muller and Valencia, 1951; Muller, 1954), other features of their data departed so strikingly from all previous experience that it was thought better not to present and discuss them until confirmative or confutative data could be secured, under more rigorous conditions than before. This has now been done. The results of this later work, constituting the third series of experiments, which was conducted in 1953 in collaboration with I. H. Herskowitz, S. Abrahamson, I. I. Oster, and F. Verderosa, have turned out to be in sufficiently close agreement with those of the first two series in essential respects to indicate that the case to be presented now rests on a firm foundation.

In all three series of experiments a study was made of the frequencies of (a) translocations, (b) losses of part or all of the sex chromosome, and (c) recessive sex-linked lethals, after application of different doses of neutrons to spermatozoa. To a lesser extent, male germ cells irradiated at earlier stages were used also, in the first two series, in the study of these three effects. In addition, in the first two series, data were obtained on (d) the frequencies of visibly expressed mutations involving specific loci, both after neutron irradiation of male and of female germ cells, in different stages.

For comparison, experiments were also carried out with X-rays. These were worked on by the same personnel and utilized exactly the same ge-

*The first two series of experiments herein reported were supported by a series of grants, designated as EG-9D-II, from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council, while the third series of experiments was supported both by these grants and by grants from the Atomic Energy Commission, designated as AT(11-1)-195).

[†]Contribution No. 552.

netic techniques and breeding systems as the first two neutron series, except that the fourth insemination period from which offspring were taken for testing extended from the 10th to 14th instead of from the 18th to 22nd day after irradiation. Two irradiations, each of 4000 r delivered at 135 K V peak and filtered through 1 mm. Al, were given at Indiana University, on July 6 and Aug. 24, 1949, respectively.

The present paper deals with the work on translocations.

2. NEUTRON IRRADIATIONS

All the neutron irradiations were carried out at the Oak Ridge National Laboratory. The source of neutrons for the first two series was the pile (utilizing uranium fission), and that for the third series the 86" ORNL cyclotron (utilizing the bombardment of Be by 22 Mev protons), an instrument discussed by Livingston (1952), Sheppard and Darden (1954), and Baker and von Halle (1954). With the latter instrument, the same set-up for the irradiation was used (employing 2-inch thick lead containers) as that employed by Kirby-Smith and Swanson (1954) and by Baker and von Halle (1954) (but not described by them). The first two series of irradiations were carried out on September 19 and November 9, 1949, respectively, and the third on March 31, 1953. On each occasion two doses were used: on the first, 250 and 500 n, presumably representing about 625 and 1250 reps; on the second, 300 and 600 n, or presumably 750 and 1500 reps; and on the third, 110 and 440 n, or presumably 275 and 1100 reps, these last determined by more advanced methods (see Sheppard and Darden, 1954).

The two doses given on each of the three occasions were of the same intensity but different durations, bearing the ratio 1:2 on the first and second occasions and 1:4 on the last. In order to insure that the relative doses on the last occasion should be precisely 1:4 for the averages of the low-dose and high-dose flies, no matter what the absolute doses may have been, a special precaution was taken. This consisted of dividing the time during which the high-dose flies were treated into four intervals, all receiving as nearly as possible the same one-quarter dose, and of having one quarter of the low-dose flies of each type present, along with the high-dose flies (but in different gelatin capsules of course), during each of these intervals. The low-dose flies from the four different intervals were thoroughly mixed together before they were set out in cultures. Thus, even if the dose had fluctuated from interval to interval, the average dose received by the low-dose flies must have been just 1/4 that received by the high-dose flies.

3. TIMING OF BREEDING IN RELATION TO IRRADIATION

In the first two series of neutron experiments the irradiated males at the time of their exposure were from about 3 to 8 days old, counting from the time of eclosion. They were placed in mass cultures with five successive sets of virgin females, for the intervals 0-2, 2-6, 6-10, 10-18, and 18-22 days after irradiation. The females of the fourth set (10-18 days) were discarded, having been used merely to effect continued discharge of sperm by

the males. The females of the other four sets, after separation from the males, were transferred to fresh cultures ("broods") every four days until, after 12 days, they no longer laid fertilized eggs, and the data derived from all the cultures of any given set of females were combined. It is to be noted that the results from the successive sets of females represent the effects of the irradiation of male germ cells which were successively further removed from maturity, or at least from ejaculation, at the time of their exposure, and that in the case of the last set the majority if not all of the male germ cells used must have been spermatogonia when irradiated.

Following Luning's (1952a-c) findings of the marked differences in the mutagenic effects of X-rays on male germ cells of the first to seventh and the seventh to eleventh days before ejaculation, and on the germ cells of males of different ages (including the observation that older males are apparently more heterogeneous than young males in regard to sperm ejaculated shortly after irradiation), and the subsequent finding by Muller, Herskowitz, Abrahamson and Oster (1954) that sperm heterogeneity of these types results in selective effects of radiation which obscure the mutation frequency-dosage relation (see section 7), the suspicion arose that the results of the first two of the present series of neutron experiments might have been subjected to these same sources of error, inasmuch as the males at irradiation had been of such mixed ages, and had included some individuals in their second week of imaginal life. This was the chief reason why a third neutron series was undertaken, and it led to the choice of a group of males having, as nearly as was then feasible, maximum homogeneity in regard to the stage, at irradiation, of those germ cells which were to be followed up. For this purpose, all the males used were between 28 and 78 hours old at irradiation, and they were allowed to inseminate females for a period of only 28 hours after irradiation. They were then discarded, while the females to which they had been mated were passed through two more "broods," of 3 and 4 days duration. However, the first brood provided a nearly sufficient number of offspring, and (except in the case of some controls) the remainder were taken from the third brood. Regardless of their brood of origin, however, all the offspring used must have been derived from male germ cells which at irradiation were at a stage not more than $2\frac{1}{6}$ days prior to ejaculation.

Since the stage of development of the germ cells depends upon temperature as well as upon time, the parent flies were kept at 25°C, with a fluctuation of only about 0.5°C, except for the period of their trip to Oak Ridge. During that time, occupying not more than two days before and two days after irradiation, their temperature fluctuated between about 20°C and 25°C on each occasion. This somewhat lower average temperature has the effect of making the ages of the males at irradiation, as expressed in terms of the ages of males of the same stage which had been kept at 25°C continuously, slightly less than stated above. Moreover, their differences in age, expressed in the same terms, become correspondingly less, as do also the lengths of the intervals between irradiation and insemination.

4. METHODS OF TESTING FOR TRANSLOCATIONS

The genetic method used for the detection of translocations in the first two series was the simple one of crossing irradiated wild-type (Oregon-R) males with females homozygous for the recessive markers *bw* and *e* on their second and third chromosomes, backcrossing the F_1 males individually to females like their mothers, and then examining the F_2 of each culture for the presence or absence of all classes representing recombination of chromosomes. Theoretically, this procedure should allow the detection, in F_2 , of all viable, fertile cases of translocations connecting chromosomes II with III, or connecting Y with either II or III, except those rare cases involving only such tiny transfers that both complementary aneuploid recombinants could survive. However, special skill is required for recognizing many of the Y-II and Y-III types, since in these cases one class of recombinant (that having an autosomal duplication along with a deficiency of part of the Y) is frequently (in 25-30 per cent of cases) viable, and such cases are more likely to be overlooked. No special effort was made, in the first two series, to pick up all such cases. For this reason and because most Y chromosome translocations are lost through the male sterility engendered by them (except when special genetic mechanisms are used to avoid this), the data here recorded for these series concern themselves mainly with those translocations which connect the two major autosomes.

In the third series, a single type of parental cross served simultaneously to provide the offspring needed for all three kinds of tests there made: namely, for translocations, chromosome losses, and lethals. This parental cross was between irradiated males of composition $sc^8.Y/ac\ apr$ (there being no mutant markers in their autosomes) and virgin females of a stock, especially constructed for the purpose, having the composition $y\bar{q}-s.Y^S/y\bar{sc}^{S1} B / In49\ v; cn\ bw; e$ (the autosomal markers *cn bw* and *e* being homozygous). For detecting translocations, the phenotypically wild-type F_1 males, of composition $sc^8.Y/y\bar{q}-s.Y^S; cn^+ bw^+/cn\ bw; e^+/e$, were individually backcrossed to virgin females homozygous for *cn bw* and *e*. The combination *cn bw* (with the genes for cinnabar and brown, both in chromosome II, resulting when together in unpigmented eyes) gives an even more conspicuous means of recognizing the derivation of the second chromosome in this cross than does the *bw* marker alone, as used in the first two series, but otherwise the method of identification of the translocations is the same as before.

In the third series, special pains were taken to "spot" the translocations involving the Y as well as those connecting II with III. Moreover, the genetic technique used in this series was adapted to finding from two to three times as high a frequency of Y translocations (for any given dose) as could have been found in the first two series. This is because in the first two series, as in all ordinary crosses, in which the X chromosome used has no part of a Y attached to it, approximately two thirds of the translocations of Y chromosomes result in male sterility, because of position effects on fertility genes of the Y^S or Y^L arms (Neuhauss, 1939). However, in the

third series, the F_1 males had Y^S attached to their X, and sterility caused by recessive changes affecting fertility genes of the Y^S portion of their irradiated Y chromosome would thereby be "covered." There would still be sterility caused by translocations of the Y^L arm. These comprise, according to Neuhaus, about half of the cases which would have been sterile in the presence of an ordinary X. However, to compensate for this deficit in the frequency of recognizable translocations, the irradiated Y chromosome of the third series, being of the type known as " $sc^8.Y$ ", has attached to or inserted in its Y^L arm a portion of the X chromosome, derived from the left end of an X chromosome of scute-8 structure. This extra material includes a small piece of the left distal end of the X (containing y^+ , the normal allele of yellow) and a considerable length of heterochromatin, originally derived from the proximal region of an X chromosome to the left of the centromere. This attached portion of the X has a length at mitosis, and possibly a susceptibility to becoming broken and forming translocations, which is not very different from that of the region of Y^L which gives sterile males. Therefore the frequency of fertile translocations of the $sc^8.Y$ in the third series is likely to be similar to the total frequency of translocations of an ordinary Y, and perhaps three times the frequency of fertile translocations of an ordinary Y that is in company with an ordinary X.

In all series, cases which seemed doubtful were bred further (with the exception of a very few unbreedable cultures which were classified as in effect sterile), until a definite decision could be reached as to whether a translocation was present and, if present, as to the category in which it belonged.

5. RESULTS FOR TRANSLOCATIONS BETWEEN AUTOSOMES

Table 1 shows the numbers of F_1 males tested (steriles being excluded) for translocations, for each dose in each series, and the numbers of II-III translocations found among them, while Table 2 shows these results as per

TABLE 1
RAW DATA ON TRANSLOCATIONS OF TYPE II-III

(Numerator of each fraction denotes number of translocations, denominator total number of fertile test-cultures.)						
Series of Exp's	Estim. reps	Days after irradiation at which insemination occurred.				
		0-2	2-6	6-10	10-14	18-22
III	275	87/4344				
I	625	31/876	41/706	33/426		2/526
II	750	50/932	44/987	49/874		0/383
III	1100	89/1319				
I	1250	42/456	34/402	29/240		1/264
II	1500	45/396	34/384	36/402		5/416
X ray (a)	4000 r	46/343	56/487	67/446	32/231	
X ray (b)	4000 r	20/169	25/274	25/210	17/219	

(For spaces left blank no data were obtained.)

TABLE 2
FREQUENCIES OF II-III TRANSLOCATIONS

(Insemination periods of 0-2 and 2-6 days combined.)
(All frequencies per rep given are to be multiplied by 10^{-6} .)

Series of exp's	Estim. reps	Days after irradiation at which insemination occurred.							
		0-6		6-10		10-14		18-22	
		observed per cent	freq./rep ($\times 10^{-6}$)	observed per cent	freq./rep ($\times 10^{-6}$)	observed per cent	freq./rep ($\times 10^{-6}$)	observed per cent	freq./rep ($\times 10^{-6}$)
III	275	2.01 ± 0.21	73 ± 8						
I	625	4.55 ± 0.53	73 ± 8	7.7 ± 1.3	124 ± 21			0.4 ± 0.3	6 ± 4
II	750	4.90 ± 0.49	65 ± 7	5.6 ± 0.8	75 ± 10			0.0	0
III	1100	6.75 ± 0.70	61 ± 6						
I	1250	8.86 ± 0.97	71 ± 8	12.1 ± 2.1	97 ± 17			0.4 ± 0.4	3 ± 3
II	1500	10.11 ± 1.07	67 ± 7	9.0 ± 1.4	60 ± 10			1.2 ± 0.5	8 ± 4
Average freq./rep			68 ± 3		$89(\pm 8)^*$				4 ± 3
X (a)	4000 r	12.30 ± 1.14	31 ± 3	15.0 ± 1.7	37 ± 4	13.8 ± 2.3	34 ± 6		
X (b)	4000 r	10.17 ± 1.44	25 ± 4	11.9 ± 2.2	30 ± 6	7.8 ± 1.8	20 ± 5		
Average freq./r			28 ± 2		$34(\pm 4)^*$		$27(\pm 4)^*$		

* All errors given are standard errors calculated on the basis of random sampling. However, the 6-10 day and older averages are subject to a much larger error than this, as shown by the fact that the values for the different series have variances much greater than those of random sampling. Presumably the additional variance was caused by differences in the timing of breeding, i.e., in the ages of males and of sperm used in relation to irradiation, between series I and II.

cents, with their standard errors, and also as frequencies per rep, in units of 10^{-6} . So, for example, there were 89 translocations of type II-III found among 1319 F_1 males tested, derived from fathers given 1100 reps in series III, as shown in Table 1. This represents a frequency of 6.75 per cent, as shown in Table 2, and when this value is divided by 1100, so as to ascertain the frequency in relation to each rep received, a figure of 61×10^{-6} is obtained.

In Table 2 the results from the first two insemination-periods (0-2 and 2-6 days after irradiation) were combined in order to obtain more representative numbers. In only one pair of these cases (that for the 0-2 and 2-6 day results with 625 reps) did a difference between corresponding figures for these periods verge on the significant, while, in general, there was no consistent direction of difference between the results for the two periods, either in the case of the translocation data or of those for the other types of changes investigated. Moreover, according to Lünig's findings (although not all investigators agree in this) these first two periods are to be expected to give substantially the same results. On the other hand, the frequencies from the 6-10 day period, although highly variable (presumably because the timing and conditions of breeding had not been so accurately controlled in these two series), give on the whole the impression of being higher than those for 0-6 days, as would also be expected on the basis of Lünig's work, and this impression is to receive confirmation when the other types of changes are examined (see other papers of this group).

As for the last period (insemination 18-22 days after irradiation), the results agree with the long known principle that far fewer chromosome changes are obtained after the irradiation of spermatogonia than of spermatozoon or near-spermatozoon stages. Yet the reduction in the frequency with which translocations are induced is not nearly as great as it appears to be, when allowance is made for the loss of one half to three quarters of the eucentric translocations induced in gonial stages, by reason of the passage of the complementary components of the translocations to different descendant cells—a contingency which is much less likely (although still not very infrequent) in the case of translocations induced in spermatozoa. This somewhat higher obtained than anticipated frequency for spermatogonia should be considered in connection with the similarly higher obtained than anticipated frequency of gross structural changes induced by X-rays in oocytes, which has recently been reported by Herskowitz (1954).

A point of major interest in the results is the fact, evident in Table 2, that with 0-6 day inseminations there are no significant differences in the yield of translocations per rep between any of the doses of neutrons used. Even the largest differences, as between the results for 625 and 1100 reps, are only 1.2 times the standard error which random sampling would produce. The chance of obtaining a difference as great as this is more than 1 in 5, and since there are only two so large among 15 differences between the 6 values taken two at a time, they are even less frequent than was to be expected from random sampling. Moreover, the difference between the

results for the lowest and highest doses is only .6 of the standard error expected for this difference on random sampling, although these two doses differ by a factor of nearly $5\frac{1}{2}$.

In view of the differences between the stocks used, the timing of the breeding, the personnel engaged in the work, the types of irradiation and the methods of dosimetry employed, this agreement, over so wide a range, is surprising. It leads us to infer that the results in the first two neutron series (and in the similarly conducted X-ray series) were not significantly affected by sperm selection. It also allows us to accept with some confidence the average result of $68(\pm 3) \times 10^{-6}/\text{rep}$, for translocations connecting chromosomes II and III when fast neutrons are applied to mature *Drosophila* spermatozoa. This is however a figure radically at variance with (far higher than) the figures derivable from the data reported by all previous investigators of the subject (see section 7).

A corollary of the comparative constancy of translocation frequency per rep, over the range studied, is the approximate proportionality of the frequency within this range to the neutron dose, as shown in figure 1.

It is true that this proportionality principle had seemed to follow from the work of Catsch, Peter and Welt (1944, but known to us only after our first two series of experiments had been completed), from those of Eberhardt (1943) and also from the first two series of the present experiments taken

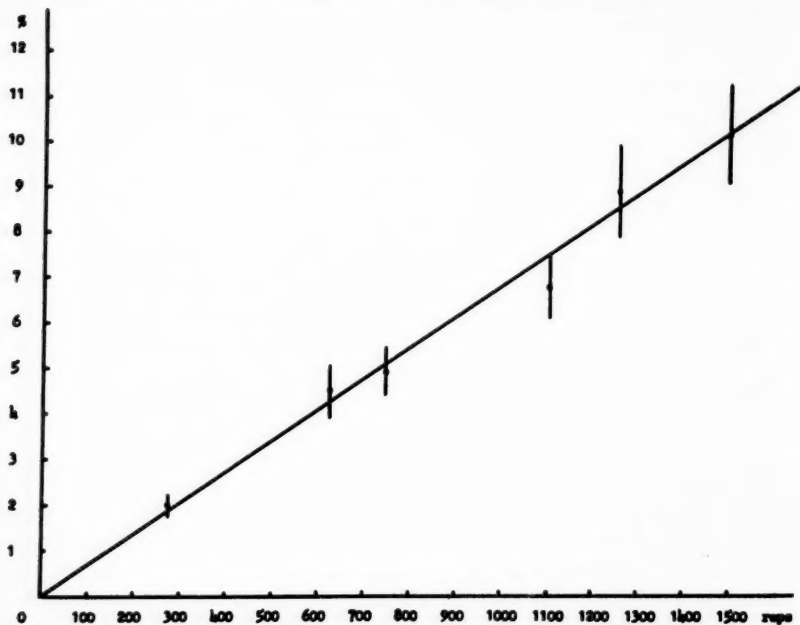


FIGURE 1. Graph of frequency (%) of translocations of type II-III (ordinate), in relation to dose of neutrons in reps (abscissa). Standard errors are given as vertical lines. (Frequency of virtually 0 for 0 reps established by other work.)

by themselves (as stated by Muller and Valencia, 1951). However, as explained in section 7, the frequencies studied by Catsch *et al.* were so low, extending only to 4 per cent, as to raise doubts concerning the applicability of the proportionality principle to higher frequencies, and doubts from another angle were raised by the eight-fold discrepancy between the mutagenic efficiency of neutrons shown by their results and ours. Reasons for doubting the validity of the results reported by Eberhardt, on translocations of the fourth chromosome, have been presented elsewhere (Muller, 1954). Moreover, as mentioned in section 3, the first two of our own series of experiments (and possibly those of Catsch *et al.* and of Eberhardt as well), did not have the timing of breeding of the irradiated flies controlled in such a way as to minimize the possibility that the observed frequency relations had been falsified by a kind of genetic selection. Only in the third series, carried out after this objection was realized (Muller, 1954), was this type of control instituted. It is therefore the results from this series, and their substantial agreement with those of the two preceding series, which now allow us to accept the proportionality principle, at least as a close approximation over the whole range studied, and also the figure obtained for efficiency per rep.

It should be explained that the way in which the use of older males for irradiation, and of sperm (even of males which were young at irradiation) ejaculated as long as a week after irradiation, obscures the frequency-dosage relation is by reason of these sperm being heterogeneous in regard to their susceptibility to having chromosome breaks produced in them. The more susceptible sperm have a higher frequency of translocations but more of the zygotes derived from them are killed off by chromosome breaks of "dominant lethal" types (mainly isochromatid-bridge cases). Therefore the surviving offspring show a lower frequency of translocations than that which was originally present among the zygotes derived from the heterogeneous group of sperm before selective death had occurred. The higher the dose, the stronger is this selective effect which favors survival from the less susceptible sperm, and the lower, in consequence, is the observed translocation frequency, relative to the frequency at which translocations had actually been induced in the mixed lot of cells. This makes the observed frequency-dosage curve less steep than the actual in such cases.

In fact, in a test of this effect on lethals produced by X-rays the observed frequency for 4000 r was found to be reduced to less than four tenths of the value, relative to that for 1000 r, which must have obtained in the material prior to selective survival (Muller, Herskowitz, Abrahamson and Oster, 1954). Under these circumstances, then, data showing a simple relation of proportionality may be suspected of having been derived from material in which, before selection, the frequency of the changes in question had been proportional to a higher power of the dose than one, such as the $3/2$ power known to obtain for translocations induced by X-rays. It is this interpretation of our results which has been refuted by the third series.

6. RESULTS FOR TRANSLOCATIONS INVOLVING THE Y CHROMOSOME

Table 3 gives the results in the third series for all detected classes of translocations, as obtained by each of the three groups of observers. There are no significant differences between the frequencies found for any given class by the different personnel, a fact serving to increase confidence in the reliability of these results. As for the observed translocations involving the $sc^8.Y$ chromosome with II and/or III, which as noted in section 3 may be expected to be similar in frequency to all translocations that involve an ordinary Y with II and/or III (including those which give sterile males), it is of interest to compare their frequency with that of translocations connecting II and III.

TABLE 3
TRANSLOCATIONS OF ALL OBSERVED TYPES IN 3RD SERIES OF EXPERIMENTS

reps	Observer-group*	II-III	Y-II	Y-III	Y-II-III	Total fer.
275	I H H	29	8	9	0	1540
275	H J M	39	6	20	1	1711
275	I I O	17	8	11	1	1193
275	Sums	85	22	40	2	4344
275	%s	$1.95 \pm .21$	$0.51 \pm .11$	$0.92 \pm .14$	$0.05 \pm .03$	
1100	I H H	27	7	12	4	407
1100	H J M	20	7	11	4	398
1100	I I O	32	10	19	2	514
1100	Sums	79	24	42	10	1319
1100	%s	$5.99 \pm .68$	$1.82 \pm .37$	$3.18 \pm .50$	$0.76 \pm .24$	

*I H H denotes group consisting of I. H. Herskowitz and S. Abrahamson,
H J M denotes group consisting of H. J. Muller and F. Verderosa,
I I O denotes I. I. Oster, with collaboration of E. Lasley.

The precise numbers of translocations of the different possible types cannot be determined from our data because any translocation of the small class designated as Y-II-III, involving all three observed chromosomes at once, could consist either of a simultaneous (1) Y-II and II-III, (2) Y-III and II-III, (3) Y-II and Y-III, or (4) a triple exchange of rotational nature involving one break in each of the three chromosomes, e.g., with attachment of one piece of Y to a piece of II, the other piece of II to a piece of III, and the remaining piece of III to the remaining piece of Y. However, the approximate numbers of translocation-combinations of the first three of these types can be calculated, in so far as these represent combinations whose component translocations were produced by independent hits, i.e., by separate proton tracks. The principle involved is that those members of any given one of these three types of translocation-combinations which have been produced in this way would occur with a frequency, xy , which was the product of x , the total frequency of occurrence, by a separate proton track, of one of the kinds of component translocations, by y , the corresponding

value for the other component. Although x and y themselves are not directly given by the data, the values for $x - xy$ and for $y - xy$ are given, being the frequencies for the translocations arising singly, and from these values it is readily possible to solve for x , y and xy . (It is true that strictest accuracy would require all three unknowns, x , y , and z , representing Y-II, Y-III, and II-III, to be reckoned with simultaneously, but as the results from this more complicated method, in cases involving such low frequencies as those here obtaining, would, despite the greatly increased labor necessary, be very nearly the same as by the approximation method here presented, this extra refinement has not been adopted.)

By following the indicated procedure, it was found that with 275 reps the frequency of Y-II translocations calculated to be produced by separate ion-tracks from those of other translocations was 0.53 per cent, while the corresponding frequency of Y-III's was 0.93 per cent, and that of II-III's 1.99 per cent. With 1100 reps the Y-II's were reckoned to form 2.00 per cent, the Y-III's 3.44 per cent, and the II-III's 6.31 per cent. Thus with 275 reps the Y-II's and Y-III's taken together were .73 times as numerous as the II-III's while with 1100 reps they were .86 times as numerous. Since the difference in relative frequencies at the two doses is not statistically significant, and since there is no ground for expecting it to be, these results may be combined, to give a frequency for Y translocations of both kinds which is .8 that of the II-III's. This is not far from (although significantly below) the value 1.0, indicating equality, which has sometimes been used for the relative frequency of translocations of ordinary Y's (including those causing male sterility) as compared with II-III's, calculated on the assumption that the Y breaks about as often as any of the four arms of the major autosomes and that union between the broken ends of different chromosomes is random. However, the non-randomness of union is here shown by the unexpected result that the Y-II's were at each dose only about 0.56 times as numerous as the Y-III's. Their numbers, for the combined data on both doses in the third series, are reckoned to be 48 and 86, respectively. There are various conceivable reasons for the differential union thus shown, but in the absence of further evidence it does not appear useful to discuss them here.

When the same methods were used in connection with the results of the first two series, obtained from the first three insemination periods (extending from 0 to 10 days after irradiation but utilizing only translocations which gave rise to no viable aneuploids), it was calculated that there were about 170 translocations connecting Y with II or III and 437 connecting II with III, a ratio of nearly 0.4:1, or nearly half of that found for these types in the third series. As indicated in section 4, a value only a third as great as that of the third series, instead of a half, would have been expected on the assumption that two thirds of the Y translocations of the first two series were lost through male sterility—in fact, less than a third, in view of the exclusion, from the calculations of the first two series only, of translocations which gave rise to viable aneuploid recombinants. This apparent discrepancy may indicate that the attached scute-8 section of the X (in-

cluding its heterochromatin), present on the $sc^{\Delta}Y$, is not as subject to translocations as is the Y^L arm (see section 3). As for the numbers of Y-II's and Y-III's in this material of the first two series, they were reckoned to be 64 and 72, respectively, giving a ratio of nearly .9:1, as compared with the .55:1 of the third series. Whether this difference was caused by the exclusion, from the data of the first two series, of Y-translocations which gave viable aneuploids, or by the difference in the type of Y used, or in the portions of the Y whose breakage was allowed (by the special genetic techniques employed) to produce male-fertile translocations, or by some other circumstance, must here be left undecided.

The question whether the Y-II-III translocations represent merely the accidental concatenation of two or more two-break translocations has been tested by comparing, for each series and dose, the number of observed Y-II-III cases with the number calculated as the expectation if they had all arisen in this accidental way. Only the results from the 0-2 and 2-6 day insemination periods were used, to avoid the influence of sperm heterogeneity and selection. In all, 32 cases of Y-II-III translocations were observed in these lots, as compared with an "expectation" of somewhat less than 14. It is therefore evident that there is a source of these cases other than that provided by the coincidence of two or more essentially separate translocations. Conceivably, such a result could be produced if there were a tendency for a sperm which had one translocation induced in it to have another induced in it also (i.e., if translocations were correlated in their occurrence), but this would imply that the sperm were distinctly heterogeneous in their mutabilities and evidence has already been given which indicates that this was not the case in the present material. Alternatively, it must be inferred that the excess of Y-II-III cases over the expected number consists of translocations more than two of whose component breaks resulted from the same proton track. Although a few of these could be four-break translocations of one kind or another it is to be expected on grounds of probability that most of them would be three-break translocations, of the rotational nature previously described. It can be calculated, from the excess of Y-II-III cases, that whenever a translocation involving the Y and a major autosome is produced by a proton track, there is a chance of roughly 10 per cent (between 5 and 15 per cent) that there will be a break in still another chromosome, caused by the same proton track, with resultant production of a Y-II-III translocation.

Table 4, giving the frequencies of all observed types of translocations involving the Y, as found for the different series and doses, among the offspring of the earlier (0-6 day) inseminations, may be used for judging in how far the Y translocations agree in their frequency-dosage relations with the proportionality principle found to hold for those of II-III type. These data are not entirely independent of the data for the II-III type, because of the inclusion in both of the Y-II-III cases, but as the latter comprise only about 20 per cent of Y-translocations in Series I and II and 10 per cent in Series III this overlapping would be insufficient to obscure a distinct dif-

TABLE 4
FREQUENCIES OF ALL OBSERVED CASES OF TRANSLOCATION OF Y
INCLUDING TYPES Y-II, Y-III, AND Y-II-III

Types of Y and X used	Ser.	reps	numbers trans/ totals	%	frequency per rep ($\times 10^{-6}$)
Y/X	I	625	25/1388	1.80	28.8 ± 5.8
Y/X	II	750	39/1901	2.05	27.3 ± 4.4
Y/X	I	1250	26/844	3.09	24.7 ± 4.9
Y/X	II	1500	13/771	1.69	11.2 ± 3.1
sc ^a .Y/X.YS	III	275	64/4344	1.47	53.5 ± 6.7
sc ^b .Y/X.YS	III	1100	76/1319	5.76	52.3 ± 6.1

(Results from inseminations of 0-6 days for Series I and II and from 0-2 days for Series III. Only Y translocations which gave no viable aneuploids are here recorded for series I and II, but all Y translocations are recorded for series III.)

ference in the frequency-dosage relation. It will be seen that the results of the first two series include one serious case of disagreement, that for 1500 reps, but that the other three frequencies derived from these two series are in good agreement. In view of the fact that, as explained in section 4, the results for Y-translocations in Series I and II are not expected to be very reliable, the disagreement is not as surprising here as the agreements. Moreover, it is in the wrong direction for a frequency-dosage relation like that which obtains with X-rays. More significant are the results of the third series, which are presented apart from the rest because of their higher reliability for Y-translocations and because the genetic technique allows a so much higher frequency of them to be detected. These results, despite their spanning a four-fold range of dose, are strikingly in line with the proportionality principle.

In none of the above calculations has the attempt been made to allow for the deviation from proportionality caused by the concatenation of two or more effects of the same type in the same case, e.g., two II-III's at once (the so-called "saturation effect"), because these deviations may be reckoned in all these cases to be a good deal smaller than the errors of sampling. Nevertheless, the existence of this source of error would give rise to a trend which resulted in the observed frequencies for the higher doses running, on the average, somewhat below that calculated on simple proportionality, and there seems to be evidence of this trend in most of our data.

7. INTERPRETATIONS

At low enough doses of ionizing radiation of any type the frequency of translocations or of any other induced changes is necessarily proportional to dose, because a cell is hardly ever traversed by more than one track within which ionization occurs, so that the frequency of the changes is simply proportional to the frequency of cells containing such a track. The higher the density of ionizations per unit of track, the higher is the level

of dose below which this mechanism operates to enforce the proportionality of translocation (or other mutation) frequency to dose. Thus the question arises, is the proportionality found in the present experiments referable to this cause, i.e., was the dose low enough for this effect, in consideration of the specific ionization of the tracks of the protons released by the neutrons used?

One way to attack this question would be to determine the combined length of ionizing tracks traversing a unit volume, e.g., 1 cubic micron (μ^3) of the biological material at a given dose, to convert this into the combined track length for a volume equal to that of a sperm cell, and then to divide this value by the average length of each track within the sperm cells. This would give the average number of tracks per sperm cell, and their average distance apart. A figure for the combined track length per μ^3 of protons arising from irradiation of exactly the type here used does not appear to be readily available. However, according to Lea (1946, Table 18), there is at a dose of 1000 reps a combined track-length of about $2\frac{1}{4}\mu$ per μ^3 of volume in the case of protons produced by a D+D reaction of 0.3 Mev deuteron energy. The protons themselves here can be reckoned from their track range (see Lea, Table 13) to have an energy of not quite 2 Mev, on the average. Taking the volume of the sperm nucleus at a minimum of $0.37\mu^3$ (by assuming a cylinder with a diameter of only $\frac{1}{4}\mu$ and a length of $7\frac{1}{2}\mu$), there would be combined track length of just 1μ within a sperm nucleus. Taking the diameter of this cylindrical nucleus as $\frac{1}{4}\mu$, this would be the average length of one individual track within the sperm, and there would have to be four tracks per sperm, on the average, to give the combined track length of 1μ .

Since, according to Sheppard and Darden (1954), the peak neutron energy of the ORNL cyclotron is only between 1 and 2 Mev, the energy of the protons of their release would probably average somewhat less than 1 (but considerably more than $\frac{1}{2}$) Mev. Hence the combined track length, as well as the number of tracks per sperm, might in this case be as low as a quarter of that above reckoned. It would then be doubtful whether the average sperm had more than one track produced in it by 1000 reps. Nevertheless, even if the average number of tracks per sperm at 1000 reps were as low as 1, there would be such a considerable frequency of sperm traversed by more than 1 track (26 per cent, on a Poisson distribution) that the frequency-dosage relation for mutational changes, in the range between 0 and 1000 reps, would be distinctly changed from proportionality, provided different tracks were free to cooperate in giving the changes studied. In the first two series, utilizing neutrons derived from uranium fission, it is estimated (see e.g. Conger, 1954) that the neutrons have an average energy of about 1 Mev, so that the proton energy would be somewhat smaller and the number of tracks per sperm somewhat fewer than in the third series.

Fortunately we have a more decisive approach to the present problem, based on observed biological effects. Counts of offspring per parent made for the third series indicate that at 1100 reps something like nine tenths of

the zygotes were killed before the imaginal stage by dominant lethal effects. More accurate studies made on egg hatchability, by Baker and von Halle (1954), allow the calculation of an embryonic death rate of some four fifths, caused by 1100 reps of neutron radiation of the same kind. Hence (if we exclude the possibility here of such effects from tracks outside the affected cell, as there are other grounds for doing) at least this proportion of sperm must have contained one or more tracks. Now, according to the theory of Muller and Pontecorvo (Muller, 1941; Muller and Pontecorvo, 1941; Pontecorvo, 1942), which has since been generally adopted (e.g. by Demerec and Fano, 1944, and by Lea and Catcheside, 1945), these dominant lethal effects are caused by chromosome breaks (usually single breaks), but the great majority of breaks restitute without causing death. Hence the .8 of zygotes which died are evidence of a frequency of breaks among the sperm several times as high as this. Virtually all sperm must therefore have had at least one break, and for this to be true the average number of proton tracks per sperm must have been well above 2, at a dose of 1100 reps of neutrons from the cyclotron.

It therefore seems safe to conclude that the "linearity" of the translocation frequency-dosage curve, at least for the third series, is not a mere reflection of a range of doses which resulted in no more than one proton track per sperm. The corollary follows that, when more than one proton track is present, any given translocation, with its two or more breaks, in the great majority of cases represents the effect of just one of these tracks and not the combined effect of two or more of them. Thus, when neutrons are used, unlike what happens with X-rays, the broken ends derived from different breaks resulting from the same track must join selectively with one another, rather than with broken ends derived from other tracks. This in turn implies a rather close spatial localization, both in the production of the breaks by the activated particles and in the union of the broken ends.

The above interpretation of neutron effects was first arrived at by Giles (1940, 1943), for the chromosomes of *Tradescantia* microspores, as a result of his finding of a proportional relationship between the frequency of structural changes in them and the neutron dose, unlike the relationship found for X-rays in this material. The possibility remained open, however, that in *Drosophila* spermatozoa the pathway from activation to breakage might extend over a much longer distance, at least in relation to the size of these much smaller nuclei, and that, even if this were not the case, the chromosomes and their pieces might become so shifted in their relative positions after fertilization, before union of pieces took place, that there could be little preferential union between broken ends derived from the same, as compared with those derived from other, proton tracks. Nevertheless, despite the great differences in the types of organisms, the cell stages dealt with, and the condition of their chromosomes, the present results prove to be the same in principle as those obtained in *Tradescantia*.

The reason why the principle of selective union of broken ends derived from the same track is evaded when X-rays are used is of course because

the greater scattering of the activations produced by X-rays allows the broken ends derived from different tracks oftener to be close enough together for union, while, complementarily, there are relatively less often cases of breaks so close together, derived from the same track. However, even with neutrons, if a high enough dose could be tolerated, the frequency-dosage curve would become concave. If a series of tests could be made of ionizing particles having differing, narrowly delimited ranges of specific ionization, to determine at what combinations of energy and dose the proportionality principle begins to be modified, conclusions could be drawn in terms of fractions of micra concerning the distance that may separate the sites of activation from those sites in the spermatozoon chromosomes between which union of broken ends will later (doubtless after some change in their relative positions) occur. At present, we can only estimate that, for this material, this distance is almost certainly less than 2μ , and probably less than 1μ .

As previously stated, the length of proton tracks within a spermatozoon nucleus must average about $\frac{1}{4}\mu$ (its diameter). Very few would be longer than two or three times this. Nevertheless, a large proportion of translocations involve breaks far removed from the chromosome ends, even when neutrons are used. Since the two breaks which participate in a given translocation induced by neutron irradiation are effects of the same tracks, the conclusion follows (unless we stretch the interpretation so as to allow the breaks to be produced at more than a micron from their instigating activations) that the chromosomes must overlap one another to a considerable extent along the length of the sperm head. This inference, which is at variance with the prevalent idea of straight end-to-end arrangement of chromosomes within the spermatozoa, has recently received support from a cytological study made to test it (Herskowitz and Muller, 1954). That the overlapping is multiple and involves more attenuation and major folding of the chromosomes than they have at mitosis, and perhaps even some interpenetration of coils like that of interphase, is indicated by the evidence, given in section 6, that a considerable proportion of translocations involves breaks in three chromosomes at once, all derived from activations of the same proton track. In this connection the related observations of Fano (1944) may be recalled, that of 25 cytologically examined structural changes induced by neutrons 6 involved more than 2 breaks, and that inversions were at least as common, in relation to translocations, as among the structural changes produced by X-rays.

Since the relative efficiencies of neutrons and X-rays in producing structural changes vary with the dose, because of the differing frequency-dosage relations for these types of radiation, it is invalid to specify these relative efficiencies, or even to state that one agent is more or less efficient than the other, or equal in this respect, as some authors have done, unless the dose or, better, the yield of changes, is specified also. We may, for example, choose doses which yield approximately 10 per cent of translocations of type II-III, as ordinarily given by some 3750 r of X-rays (Muller,

1940 and data of the present Table 1). In that case we find that, on a frequency-dosage relation of proportionality for neutrons, the same yield (10 per cent) would be obtained with 1500 reps of neutrons, according to the data herein reported. This would connote an efficiency for neutrons of $2\frac{1}{2}$ times that for X-rays, at this yield. On the other hand, extrapolating from the data of Dempster (1941), and also Demerec, Kaufmann and Sutton (1941, see also Kaufmann, 1941), neutrons would, at this level of yield, be approximately equal to X-rays in efficiency (though X-rays themselves, in the latter set of data, were only about .3 as efficient in recessive lethal production as they ordinarily have been found to be), while by extrapolation from the data reported by Catsch, Peter and Welt (1944), the efficiency of neutrons, at this yield, would be only three tenths that of X-rays. It can be calculated that for a yield of 1 per cent (ordinarily given by about 800 r of X-rays) the efficiency of neutrons would according to the present work be 5.4 times that of X-rays, according to Dempster 2.15 times and according to Catsch *et al.* about .7 as much.

As the effects for lower and lower yields were studied the efficiency of neutrons as compared with X-rays would continue to increase, until doses of X-rays were reached which were so low that even with this agent only a negligible proportion of cells was traversed by more than one ionizing particle. Below this level, the efficiencies of neutrons and X-rays relative to one another, as well as their absolute efficiencies, would remain constant.

Although the relative efficiencies of neutrons and X-rays in producing translocations would vary with the yield or dose, the relative efficiencies of neutrons themselves, as found by the techniques of different observers, would be independent of dose, so long as the proportionality relation held in all cases. Hence, at all yields and doses, as at the 10 per cent yield, the relative efficiencies of neutrons as (1) herein reported, and as reported by (2) Dempster, and (3) Catsch *et al.*, respectively, would follow the ratio 2.5:1:0.3, or, if the efficiency herein reported were denoted as 1, the ratio would be stated in the form 1:0.4:0.12.

A relatively small part of this variation in observed neutron efficiency was probably caused directly by differences in specific ionization, arising from differences in the energy of the neutrons used by the different groups of investigators. However, if this were the main source of the variation one would not expect to find the seriation in efficiency to be that above given. For although our series II probably involved a lower energy, and therefore a higher specific ionization, than the radiation used by the other investigators, and showed a higher efficiency than that found by them, nevertheless the experiments of Catsch *et al.* probably employed neutrons of lower energy (produced by the Li+D reaction) than did the experiments of Demerec *et al.* (in which a Columbia cyclotron of early type was used), yet the former showed only $\frac{1}{4}$ the efficiency of the latter, as measured in terms of their X-ray controls. Moreover, the ORNL cyclotron showed, in the present experiments, substantially the same efficiency as the pile. Even

more significant in this connection are the recently reported findings by Conger (1954) and especially those by Kirby-Smith and Swanson (1954) that even the neutrons derived from a nuclear detonation have sensibly the same efficiency in producing chromosome breaks and structural changes in *Tradescantia* as do the neutrons from the ORNL cyclotron, and the parallel finding by Baker and von Halle (1954) that in the production of dominant lethals in *Drosophila* neutrons from these two sources were at least of very similar efficiencies. It is therefore unlikely that differences so enormous as those reported by different authors for the production of translocations by neutrons in *Drosophila* were caused by differences in specific ionization, arising from differences in the neutron energy.

A related cause of some of the variation in efficiency, besides the differences in specific ionization in themselves, might be the fact that the tracks of different energies would attain maximum concentration, and peak energy absorption, at different depths in the tissues. Only for the lower energies does *Drosophila* have sufficient depth for maximum absorption to occur within its testes. But, again, the magnitude of the reported variations is greater than that to be expected from this source. Furthermore, the variation is in the wrong direction when the results of Catsch *et al.* and of Dempster are compared. It would seem, therefore, that most of the reported variation in efficiency was caused by differences in dosimetry, including the estimation or measurement of the effects of shielding, scattering, etc.

The greater efficiency of neutrons than of X-rays in producing translocations within the dosage range used in the present series of experiments, as shown by the data obtained therefrom, cannot properly be regarded as evidence, in itself, that the efficiency of neutrons is greater than that of X-rays in producing chromosome breaks. For the spatial distribution of the breaks produced by neutrons must, over much of this range at least, be more favorable for the union of ends derived from different breaks—i.e., for rearrangement as compared with restitution or isochromatid formation,—than the distribution of the breaks produced by X-rays.

Despite these restrictions of interpretation, the present quantitative information concerning the frequency of production of structural changes by neutrons, and its manner of variation with dose, is useful, when taken in connection with data on the frequency of induction of chromosome losses by neutrons, in allowing conclusions to be reached concerning the relative efficiencies of neutrons and X-rays in causing chromosome breaks. Similarly, in connection with data on the frequency of lethals, the present information makes conclusions possible concerning the relative efficiencies of these agents in the production of point mutations. These matters will be dealt with in the three succeeding papers of this group.

SUMMARY

1. The frequency of translocations between the Y, the second and the third chromosomes, induced by neutron irradiation of mature *Drosophila* spermatozoa (ejaculated within six days after irradiation), varies in linear

manner with the dose of radiation over the range studied, extending from a frequency of 2 per cent to about 10 per cent for translocations connecting the second and third chromosomes. That this is not a mere appearance of linearity, resulting from the differential production of dominant lethals in sperm of heterogeneous mutability, is made clear by the third series of experiments, in which the curve did not rise above the linear, over a four-fold range of dose, when sperm ejaculated within about one day after the irradiation of very young males, and therefore of comparatively high homogeneity, were used.

2. Since there is evidence that at the higher doses used a large proportion of the spermatozoan nuclei was traversed by more than one proton track, the linear frequency-dosage relation must mean that in this material (as in Giles' studies on *Tradescantia* microspores) the chromosome breaks are produced in the immediate vicinity—within a distance of the order of a micron or less—of the activations which instigate them, and that the breakage-ends of the chromosomes unite preferentially with those breakage-ends which had been produced within this distance. It follows as a corollary that the chromosomes overlap in their positions along the length of the sperm head, as has also been indicated on cytological grounds in a parallel study, by Herskowitz and Muller.

3. Translocations of the $sc^8.Y$ chromosome which allow fertility in the male when the X has the short arm of a Y attached to it are produced with a frequency about .8 as high as that of translocations between the second and third chromosomes. The frequency of the former is probably slightly less than that of all translocations (including those resulting in male sterility) which would be undergone by an ordinary Y chromosome, and it is about twice the frequency observed in our first two series of experiments for translocations of an ordinary Y which are fertile in the male and which at the same time do not give rise to viable aneuploid recombinants. Translocations connecting the $sc^8.Y$ with the third chromosome were found nearly twice as frequently as those connecting it with the second chromosome.

4. Translocations simultaneously connecting all three of the chromosomes studied (Y, II, and III) are produced with higher frequency than is explicable by the accidental concatenation of two or more separately produced translocations. The excess is probably composed in large measure of multiple, rotational exchanges. This excessive "coincidence" indicates that the overlapping of chromosome threads along the length of the sperm head is of multiple nature.

5. No significant differences were found between the frequencies of translocations obtained, per n unit of dose, with neutrons from the Oak Ridge National Laboratory pile and with those from the ORNL cyclotron. In both cases, there was a consistent production of translocations between the second and third chromosomes of $68 (\pm 3) \times 10^{-6}$ per rep. The value as calculated from the data published by Dempster would be approximately 27×10^{-6} /rep, while the data of Catsch et al. would give a value of 8×10^{-6} /rep. At a level of doses yielding a 10 per cent frequency of these

translocations, neutrons as observed in the present experiments are $2\frac{1}{2}$ times as efficient as X-rays in their production of translocations; with lower doses their efficiency in this respect, relative to that of X-rays, would rise, until that very low level of dosage was reached below which, according to theory, the production of translocations by X-rays also became linear.

ADDENDUM

Information has been received that recent studies, conducted by Dr. C. W. Sheppard, of the output of the ORNL cyclotron show the per-rep doses administered in our Series III irradiations (of April, 1953) to have been approximately 30 per cent higher than the originally estimated values of them, presented in our paper. In other words, instead of there having been a II-III translocation frequency of $67 \times 10^{-6}/\text{rep}$ (the average for Series III according to the data as presented in our paper), the frequency was actually $67 \times 10^{-6}/1.3 \text{ rep}$, an expression which reduces to $51.5 \times 10^{-6}/\text{rep}$. Since the II-III translocation frequency at 4000r of X-rays averages 11.25 per cent, or $28 \times 10^{-6}/\text{r}$, the effectiveness of the cyclotron radiation relative to that of the 4000r X-rays was $51.5/28$, or 1.84, instead of being 2.4 as previously calculated. Thus at a 10 per cent frequency, which is given by some 3700r X-rays and represents $27 \times 10^{-6}/\text{r}$, the relative effectiveness is about 1.9, instead of being, as stated in the paper, 2.5. In view, however, of some 10 per cent of the absorbed cyclotron radiation having been in the form of gamma rays, the effectiveness of the neutrons themselves, relative to X-rays, may be estimated to have been slightly (possibly some 5 per cent) greater than given by these figures. Although these changes are not known to apply to Series I and II, involving the pile, the question arises whether the previous apparent agreement between the results of these series, which showed an average frequency of $69 \times 10^{-6}/\text{rep}$, and those of Series III in fact indicate a one-third greater effectiveness of the pile neutrons than of those from the cyclotron in inducing translocations, or whether the amount of radiation from the pile had been similarly underestimated. In any case, however, the measurements of n units are not in question, only the factors for conversion of n units to reps. Thus the graph in figure 1 remains a straight line if the ordinates are taken to represent per- n translocation frequencies. The reader can obtain these per- n frequencies, both for the figure and the tables, by multiplying the therein reported per-rep frequencies by $2\frac{1}{2}$.

It should further be pointed out that the NXE values, even when reduced so as to fit the most recent estimate of the doses in reps, apply only to results obtained from sperm released soon after exposure in the male. As shown by results of my colleagues Abrahamson and Telfer (1954, *Rec. Genet. Soc. Am.* 23: 28-29 and 71-72, and unpublished), obtained since the present paper was written, the frequency of translocations induced in such sperm by X-rays is less than half as high as that of sperm X-rayed in the

inseminated female. Yet when neutrons are used the frequencies induced in sperm irradiated in the male are probably not substantially lower than when the sperm are irradiated in the female, if we may judge by the similarity of loss and dominant lethal frequencies in sperm of the first and second day exposed to neutrons in the male (Abrahamson and Telfer, unpublished; Baker and von Halle, 1953, P.N.A.S. 39: 152-161). Hence for sperm exposed in the female NXE for translocations is probably *less* than 1, for a 10% yield of type II-III translocations. Similar considerations apply for other kinds of mutations and, along with the dosage revision, make necessary qualification and modification of the NXE values presented by the present writer in a recent abstract (1954, Rec. Genet. Soc. Am. 23: 58).

H. J. MULLER,
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These acknowledgments apply equally to the work reported in all the papers of the present group, dealing with the genetic effects of neutrons.

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